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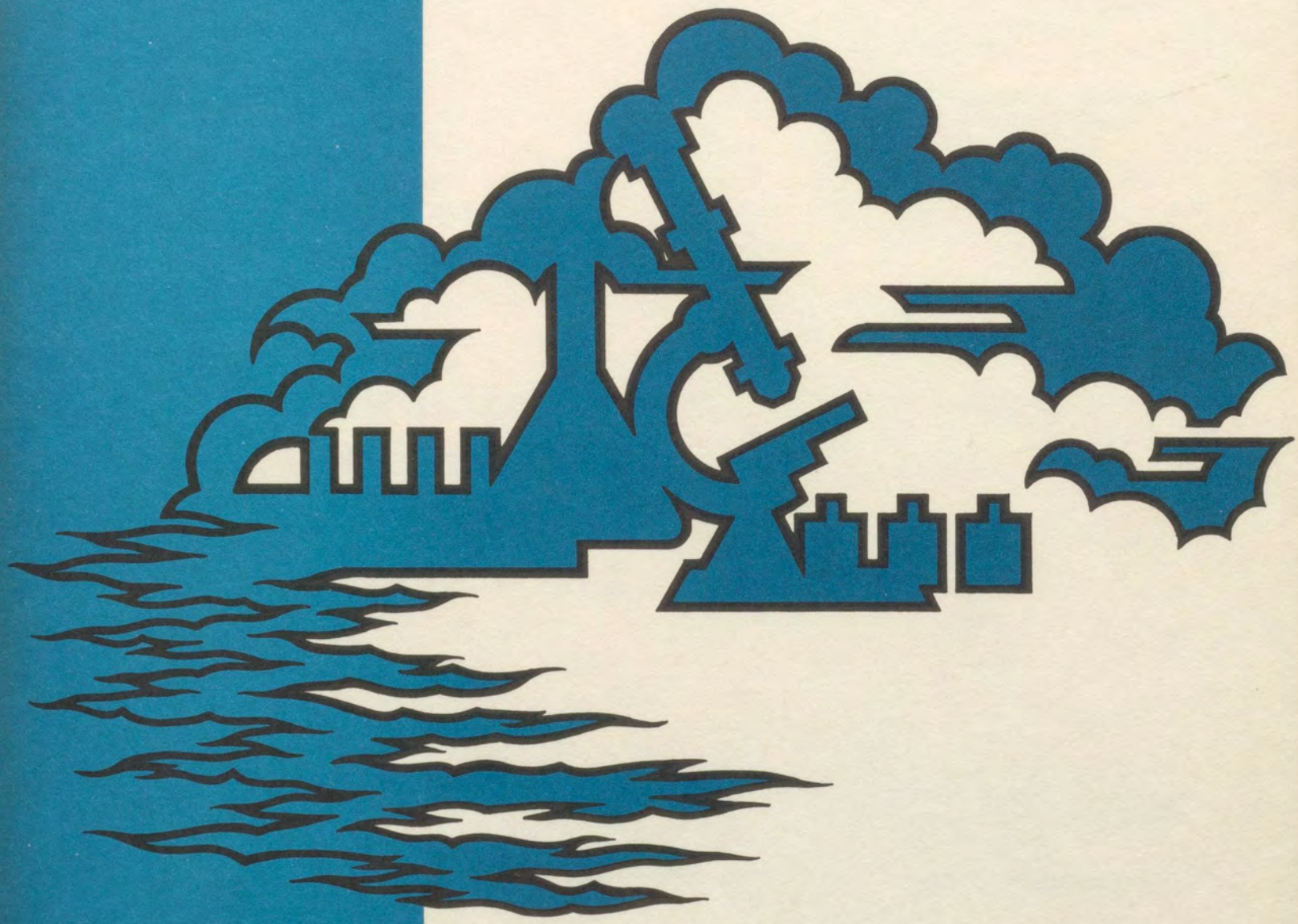

INTERNATIONAL JOINT COMMISSION

SYMPOSIUM ON STRUCTURE — ACTIVITY CORRELATIONS IN STUDIES OF TOXICITY AND BIOCONCENTRATION WITH AQUATIC ORGANISMS

Proceedings of a Symposium held in Burlington, Ontario at the Canada Center for Inland Waters, March 11-13, 1975, sponsored by STANDING COMMITTEE ON THE SCIENTIFIC BASIS FOR WATER QUALITY CRITERIA OF THE INTERNATIONAL JOINT COMMISSION'S RESEARCH ADVISORY BOARD

Structure-Activity Correlations in Studies of Toxicity and Bioconcentration with Aquatic Organisms

edited by

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NOTICE

Statements and views presented in these proceedings are totally those of the speakers and do not necessarily reflect the views and policies of the International Joint Commission or its Research Advisory Board and Committees framework. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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PREFACE

The arrangement of this document is such that the use of structure-activity correlations is first illustrated, followed by presentations on determination and application of structural parameters, and models concerned with the problems of multiple toxicity and periodic exposure. The papers were intended to be informative as well as provocative. The stimulation of discussion was a major aim in all instances.

The Editors have made free use of editorial privilege in their attempt to clarify the general discussions as well as retain informality. The participants cooperated actively in the preparation of amended remarks. A primary objective was to retain all remarks which show actual development of new ideas, illustrate the concerns of various audiences and illustrate to the general reader the potential and limitations of structure-activity correlations.

Furthermore, the participants have reviewed the Editors' attempt to clarify the conclusions and the identified research needs noted during the symposium.

A requirement, in conflict with the attainment of editorial perfection, was the achievement of an early publication date. It is believed that the value of this publication is enhanced by relatively early availability and the Editors apologize to participants and readers alike for any imperfections which may have been eliminated if time were of no consequence.

The provision of facilities by CCIW and the excellent cooperation of CCIW staff members, in particular Mrs. Irene Powell, Mr. Ian McGregor and Mr. A. R. Kirby, in expediting and tape recording the activities of the symposium is very much appreciated. These tape recordings were used to prepare the discussion section of the proceedings, and were invaluable in shortening the time span between the date of the symposium and the date of publishing.

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SUMMARY

This publication presents the proceedings of a symposium which discussed the potential of structure-activity correlations in studies of toxicity and bioconcentration of chemicals with aquatic organisms. The symposium was sponsored by the Standing Committee on the Scientific Basis for Water Quality Criteria of the International Joint Commission's Research Advisory Board and was held at the Canada Centre for Inland Waters in Burlington, Ontario, Canada on March 11-13, 1975.

The symposium consisted of formal papers on the applications of structure-activity models in laboratory testing as well as models concerned with the problems of multiple toxicity and periodic exposures. These presentations were followed by an open forum of discussion of the potentials and limitations of these techniques, the identification of immediate research needs, and recommendations pursuant to the fulfillment of these needs.

INTRODUCTION

As early as 1900, researchers observed that the biological activity of structurally related chemicals could be correlated to physical parameters of the chemicals. At the turn of the century, Meyer and Overton demonstrated that the concentration (C) of alcohols, ketones, aromatic hydrocarbons and esters causing isonarcosis in tadpoles was related to the octanol-water partition coefficient (P) by the general equation

$$\log \frac{1}{C} = a \log P + b$$

where a and b are empirically determined constants. These results become the origin of structure-activity correlations and provided the research community with one of the first techniques for modeling the biological activity of organic chemicals.

The octanol-water partition coefficient is a measure of the lipophilic properties of chemicals. The partition coefficient is simply the ratio of the concentrations of a chemical after it is allowed to equilibrate between octanol and water phases in a test tube. In general, the partition coefficient of a series of chemicals varies directly with the solubility in lipids and inversely with the solubility in water.

After the initial results of Meyer and Overton, the use of structure-activity correlations focussed on mammalian toxicology, particularly in the drug industries. Within the last twenty years, Hansch and co-workers illustrated that biological

activity could be modeled more precisely by introducing an additional term in the regression equation to model the field and resonance effects arising from the electronic distribution of the chemical. The most useful electronic structural parameter has been the Hammett σ constant which is important in the binding with biological macromolecules and perhaps the deactivation of enzymes. Hansch further demonstrated that the activity of a chemical series did not always increase linearly with the increase in partition coefficient. Rather, for extremely lipophilic members of some chemical series, the activity may actually decrease due to the binding to lipids, a mechanism known as storage detoxification. All of these factors are incorporated into the equation

$$\log \frac{1}{C} = -a(\log P)^2 + b \log P + c \log \sigma + d$$

where a, b, c and d are empirically determined constants. This regression equation has been used to correlate the biological activity of hundreds of chemical series which permit the prediction of the activity of untested chemicals by using the structural parameters of the chemicals of interest. This technique has become known as the Hansch approach.

Seventy-five years after Meyer's experiments with aquatic organisms, there is a growing awareness of the usefulness of the structure-activity correlative approach to aquatic toxicity testing. The Research Advisory Board of the International Joint Commission in accordance with its Terms of Reference established in the 1972 Great Lakes Water Quality Agreement sponsored this symposium to review the potential of the structure-activity correlative methodologies in toxicity testing with aquatic organisms and to discuss current research which employs the techniques. The purpose of the symposium was to bring to the research community a realistic outlook of the potential, as well as the limitations, of the Hansch approach for the complex problem encountered in the aqueous environment.

An essential goal of research in toxicology is the ability to predict biological effects under conditions which differ from the experimental conditions of laboratory testing. As the reader of this report will observe, the term "predictive toxicology"

has a multitude of meanings depending on the nature of the problem being faced. Many observers of this workshop on structure-activity correlations (loosely termed "predictive toxicology") came with needs such as predicting the ecological effects of chemical spills, predicting the toxicity of mixtures of chemicals in complex effluents, and forecasting potential hazards of new chemicals.

In the midst of these formidable needs, the power of structure-activity correlative methods may have seemed minuscule. However, to those concerned with modeling the toxicity of structurally related chemicals and estimating the toxicity of similar, untested chemicals to aquatic organisms, the structure-activity approach appeared as a promising tool to systematize toxicity testing and bioaccumulation studies.

STRUCTURE-ACTIVITY RELATIONSHIPS IN FISH TOXICOLOGY

CHAPTER 1

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ABSTRACT

Correlations between structural constants, properties, and biological activity of organic compounds are discussed. Relationships are presented between biological activity and water solubility or hydrophobicity, electronic or other structural constants or properties of aliphatic alcohols, alkylhydroxamic acids, organophosphates, DDT analogs, triazines, alkylvinyl sulfones, industrial chemicals, anionic and nonionic surfactants. Additional groups of compounds, suitable for such studies are discussed. The types of biological activity include acute toxicity, avoidance reactions, and accumulation, which generally increase with decreasing water solubility (increasing hydrophobicity,) and biodegradability, which increases with increasing solubility. *Para*-substituted phenols are more acutely toxic than the *ortho*-isomers, but the latter are more avoided by fish. Little systematic work on structure-activity relationships has been carried out thus far with aquatic fauna and it is likely that many useful correlations will be obtained in due course.

INTRODUCTION

Quantitative relationships between structure and properties of organic molecules, and biological activity are frequently determined in the development of drugs and pesticides, but are much less used in environmental research. Environmental contaminants may not always belong to chemically closely related groups to facilitate this type of analysis. For example, the selection of a pesticide is preceded by screening of the activity of a number of analogs and isomers and the establishment of structure-activity relationships for target and non-target species (i.e., an insect and a small mammal), but only one compound is subjected to the final toxicological screening and becomes available for environmental studies. Some pesticides and many industrial contaminants are complex mixtures of closely related compounds not easily amenable for structure-activity correlations.

Within certain limitations, quantitative structure-activity relationships may be very useful for predicting environmental properties of chemicals and may lead to summarizing and rationalizing the fast increasing amount of data on organic chemicals in the environment. The main limitation is the type of activity chosen as the basis for the correlation. A structure-activity relationship may indicate the mechanism of action of a given group of compounds, but it is not likely to predict an unanticipated type of activity.

CLASSICAL STRUCTURE-ACTIVITY RELATIONSHIPS

The structure-related constants in these relationships characterize the hydrophobic, electronic, and steric properties of molecules and the relationships have the general form given in equation (1).

$$\log \frac{1}{c} = k^{(1)} [\log P]^2 + k^{(2)} \log P + k^{(3)} \sigma + k^{(4)} E_s + k^{(5)} \quad (1)$$

where c = molar concentration, characteristic for certain activity, i.e., 96hLC50

P = partition coefficient, usually between octanol and water, a characteristic of hydrophobic (lipophilic) properties,

σ = Hammett constant, a characteristic of electronic effects,

E_s = Taft constant, a characteristic of steric effects,

$k^{(i)}$ = constants obtained by fitting equation (1) to experimental data.

The rationale behind equation (1) and some of its applications were recently reviewed by Hansch (1969). Other than σ and E_s or additional so-called free-energy constants may be used (see for example Vilceanu et al. 1972; Hansch et al. 1973), and the introduction of molecular symmetry-related constants may be worthwhile since, in general, increasing molecular symmetry leads to higher toxicity (Cohen et al. 1974). The partition coefficient P may be replaced by a related constant π (Fujita et al. 1964), or, in aquatic toxicology, at least in certain instances, by water solubility.

Partition coefficients of a large number of compounds were summarized by Leo et al. (1971), and the data can be used to estimate partition coefficients of unlisted compounds. A number of π values is also presented in this reference. Additional π values are given by Fujita et al. (1964). Numerous free-energy related constants can be found in a paper by Hansch et al. (1973).

OTHER STRUCTURE-ACTIVITY RELATIONSHIPS

More recently developed relationships such as the additive model of Free and Wilson, and quantum chemical models have been used in drug research. These techniques have not yet been applied to aquatic toxicology, and the reader is referred to a review by Redl et al. (1974) for details. This review also mentions advanced data-fitting techniques based on pattern recognition, such as cluster analysis.

APPLICATIONS OF STRUCTURE-ACTIVITY RELATIONSHIPS TO AQUATIC TOXICOLOGY

A correlation between the hydrophobic (lipophilic) character of small, mostly electrically neutral molecules, and their toxicity to aquatic fauna was recognized a long time ago and reviewed by Hansch and Dunn (1972). All the relationships have the form given in equation (2).

$$\log \frac{1}{c} = a \log P + b \qquad (2)$$

where c = active concentration, mole/ℓ, causing narcosis, immobilization, median lethality, etc.,

P = partition coefficient between octanol and water,

a,b = empirical constants, a = 0.88-1.9, b = 0.35-1.05

Addison and Côté (1973) found a linear relationship between the acute toxicity of C_7-C_{11} alkylhydroxamic acids to juvenile Atlantic salmon (*Salmo salar*), and their partition coefficients (equation (3)).

$$c = 0.344\ P^* + 2.09 \qquad (3)$$

where c = 24hLC50, mg/ℓ

P^* = partition coefficient between water and carbon tetrachloride

For a comparison of the toxicities of the individual alkylhydroxamic acids, the concentration should have been expressed on a molar basis, which would make the slope of the line given by equation (3), steeper. P^* could be converted into P (partition coefficient octanol-water) by a regression equation given by Leo et al. (1971).

Kopperman et al. (1974) obtained a relationship between the acute toxicity of phenols to *Daphnia magna*, Hansch π constant, and F and R free-energy related constants of the phenols (equation (4)).

$$\log \frac{1}{c} = 0.500\pi + 0.453F + 0.636R + 3.731 \quad (4)$$

where c = 48hLC50, mole/ℓ

The correlation is good (r = 0.978), but there are some inconsistencies and possibly errors in the values of π and the free-energy related constants.

Kapoor et al. (1973) found a linear relationship between the logarithms of the biodegradability index (ratio of polar to non-polar metabolites) of p,p'-DDT and its p,p'-substituted analogs (CH_3-, CH_3O-, C_2H_5O-, and CH_3S-) in fish, and water solubility (equation (5)).

$$\log(BI) = 0.87 \log W + 1.41 \quad (5)$$

where BI = biodegradability index (concentration of polar/concentration of non-polar metabolites)

W = water solubility, mg/ℓ

Interestingly, the correlation between the biodegradability index and water solubility is better than that between the biodegradability index and partition coefficient. Similarly, Lu (1974) found a good correlation between the ecological magnification (concentration in biomass/concentration in water) of 19, mostly aromatic compounds in fish, and water solubility (equation (6)).

$$\log(EM) = -0.4275 \log W + 2.558 \quad (6)$$

where EM = ecological magnification (concentration in biomass/concentration in water),

W = water solubility, mg/ℓ

Neely et al. (1974) derived a linear log-log relationship between the accumulation of several chlorinated hydrocarbons and aromatic compounds, and partition coefficient (equation (7)).

$$\log \frac{k_1}{k_2} = 0.542 \log P + 0.124 \qquad (7)$$

where k_1, k_2 = uptake and excretion rate constants, respectively,

P = partition coefficient octanol-water.

Equation (6) predicted correctly the accumulation of endrin and chloropyrifos, which were not used to derive the empirical constants of the equation.

Our data on the toxicity of a series of nitro- and dinitrophenols to juvenile Atlantic salmon are presented in Figure 1. It can be seen that there is a linear relationship between $\log \frac{1}{c}$ and logP for the 2-alkyl-4,6-dinitrophenols and their esters (equation (8)).

$$\log \frac{1}{c} = 0.309 \log P + 5.31 \qquad (8)$$

where c = 96hLCO, mole/ℓ,

P = partition coefficient octanol-water.

Some of the highly toxic dinitrophenols are quite widely used as herbicides and potato top killers (DNB = dinoseb, DNBA = binapacryl, DNHC = dinocap). Dinocap and the respective phenol consist of a mixture of isomers, but as equation (8) indicates, the isomerism has no effect on the toxicity of these compounds to fish. PN (p-nitrophenol) and PNC (3-methyl-4-nitrophenol) are hydrolysis products of parathion and fenitrothion, respectively. ONC (3-methyl-6-nitrophenol) may be present as the phenol moiety in some fenitrothion preparations, ON (o-nitrophenol) and P (phenol) were included for comparison.

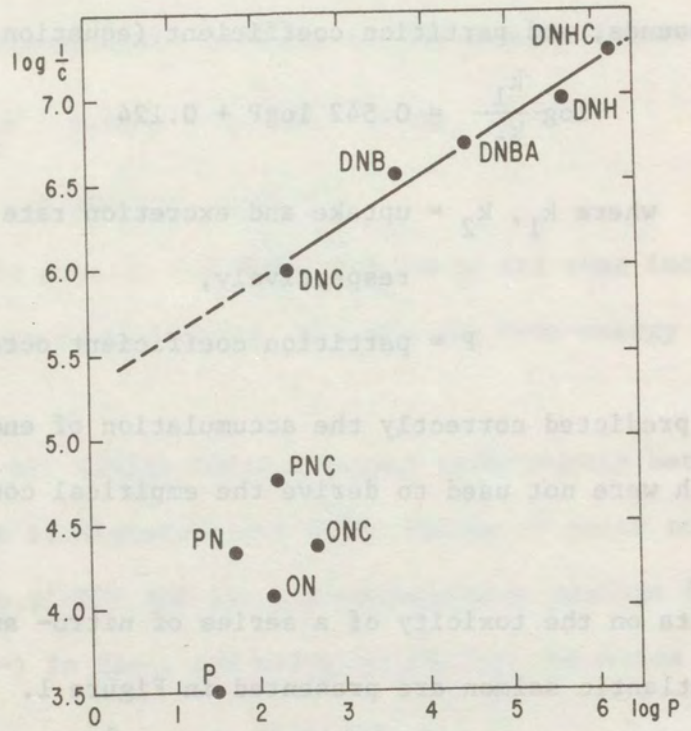

Fig. 1. Toxicity of nitro- and dinitrophenols to juvenile Atlantic salmon (*Salmo salar*).

c = 96hLC0, mole/ℓ, determined in static tests at 9°C, water hardness 14 mg/ℓ as calcium carbonate,

P = partition coefficient octanol-water.

Symbols in the Figure: P = phenol, ON,PN = o-, and p-nitrophenol, respectively, ONC,PNC = 3-methyl-6-nitro-, and 3-methyl-4-nitrophenol, respectively,

DNC = 2-methyl-4,6-dinitrophenol,

DNB = 2-*sec*-butyl-4,6-dinitrophenol (dinoseb),

DNBA = 2-*sec*-butyl-4,6-dinitrophenol 3-methyl-2-butenoate(binapacryl),

DNH = a mixture of 2,4-dinitro-6-octyl- and 2,6-dinitro-4-octylphenol,

DNHC = a mixture of 2,4-dinitro-6-octylphenol 2-butenoate and 2,6-dinitro-4-octylphenol 2-butenoate (dinocap).

The toxicity of the nitrophenols is related by equation (9) to the partition coefficient and Hammett σ constant.

$$\log \frac{1}{c} = 1.20 \log P + 2.91\sigma \qquad (9)$$

where c = 96hLCO, mole/ℓ

σ = Hammett constant, 0.78 for *para*- and 0.49 for *ortho*- nitro group.

In connection with equation (9) it should be mentioned that, due to steric interactions, Hammett constants are usually not available for *ortho*- substituents. The value of 0.49 was calculated from equation (9), derived for *para*- substituted phenols, and the toxicities of the *ortho*- isomers. Equation (9) predicts correctly the toxicity of 2,4-dinitro- and 2-methyl-4,6-dinitrophenol, which contain one *para*- and one *ortho*- nitro group ($\Sigma\sigma = 1.27$ for nitro groups).

Para- substituted nitrophenols are more toxic than the respective *ortho*- isomers. This may be a general pattern of *para*- *versus ortho*- toxicity, since the same order of toxicity was observed for *para*- and *ortho*- cresol, chlorophenol, iodophenol, and hydroxybenzoic acid (Batelle's Columbus Laboratories 1971). According to the data of Kopperman et al. (1974), p-chlorophenol was more toxic than o-chlorophenol to *Daphnia magna*, but the toxicity was reversed in the case of cresols. These authors used *para*- constants for both isomers, so that any predicted differences in toxicity were due only to differences in π. LogP values are higher for p-halogenophenols than those of the *ortho*- isomers, but the opposite is true for cresols and nitrophenols (Leo et al. 1971). On the other hand, Hansch π constants follow the same trend for halogeno- and nitrophenols and a π constant for o-cresol is not available (Fujita et al. 1964).

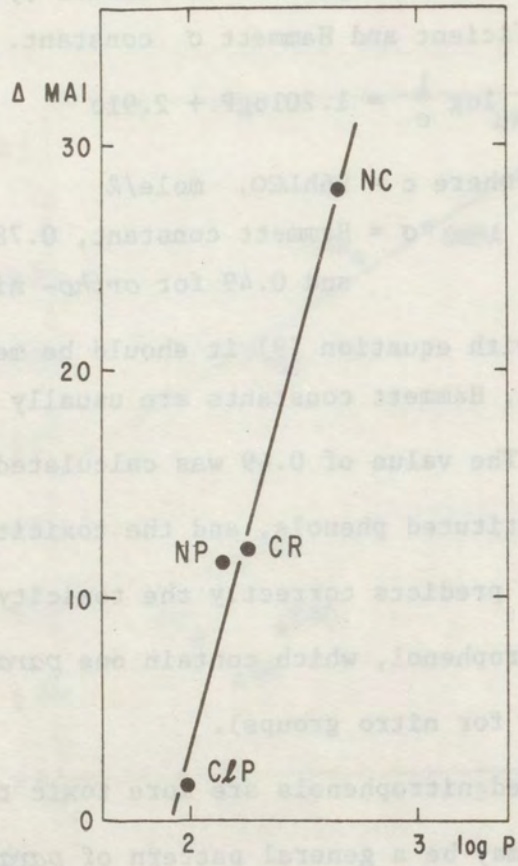

Fig. 2. Avoidance of substituted phenols by juvenile Atlantic salmon (*Salmo salar*).

ΔMAI = difference between the median avoidance index of *ortho-* and *para-* isomers. Determined in an avoidance tube (Zitko and Carson 1974).

P = partition coefficient octanol-water for the *ortho-* isomer.

Symbols in the Figure: ClP = chlorophenols, NP = nitrophenols, CR = cresols, NC = 3-methyl-nitrophenols.

Our data indicate that in contrast to their lower acute toxicity, *ortho-* substituted phenols are more avoided than the respective *para-*

isomers by juvenile Atlantic salmon. As shown in Figure 2 and equation (10), the difference in the avoidance index is linearly related to logP of the *ortho*- isomer.

$$\Delta(\text{MAI}) = 37.13 \log P - 70.45 \qquad (10)$$

where Δ(MAI) = difference between the median avoidance index of *ortho*- and *para*- isomers. The median avoidance index is the difference between the median percent time in clean water under test and control conditions (Zitko and Carson 1974),

P = partition coefficient octanol-water.

No quantitative toxicity to fish — structure of pesticides relationships are available in the literature. The toxicity of some organophosphate pesticides may be correlated with their solubility in water. According to Figure 3, acute toxicity (mostly 96hLC50 values) decreases with increasing water solubility, somewhat more steeply for the vinyl than for the mercapto phosphates, and the correlation coefficients are -0.998 and -0.655, respectively. The toxicity of several pesticides does not follow this pattern. Trichlorfon and mevinphos in the vinyl series, and phorate in the mercapto series are much more toxic than one would expect on the basis on their solubility in water, and it would be interesting to find out why. The data of Bathe et al. (1972) indicate a significant correlation between the logarithms of toxicity (96hLC50) and water solubility of 11 triazine herbicides (r = 0.820), but a similar correlation for substituted urea herbicides is not significant.

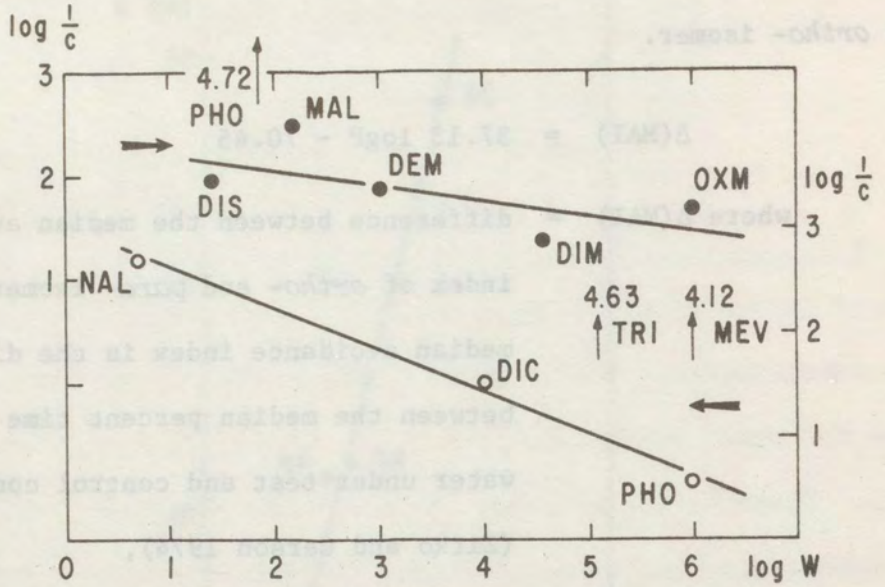

Fig. 3. Toxicity and water solubility of organophosphate pesticides.

c = 96hLC50, mole/ℓ, mostly literature data,

W = water solubility, mg/ℓ, literature data.

Symbols in the Figure: PHO = phorate, MAL = malathion, DIS = disulfoton, DEM = demeton, DIM = dimethoate, OXM = oxydemeton-methyl, NAL = naled, DIC = dichlorvos, TRI = trichlorfon, MEV = mevinphos, PHO = phosphamidon.

The correlation between toxicity and water solubility is only the first step and additional constants should be used to improve the relationship.

Because of generally low toxicity, herbicides received relatively little attention from fish toxicologists, but it is important to establish

toxicity relationships for aquatic flora. A review by Barth and Michel (1969) may be a good starting point.

Some structure-toxicity relationships have been established for surfactants. For linear C_8-C_{16} alkylbenzene sulfonates, Marchetti (1965) presents data of Hirsch, which show a linear relationship between the logarithm of toxicity and the number of carbon atoms in the alkyl chain. The function, calculated from the presented data (equation 11) differs from that given by Marchetti.

$$\log c = 6.52 - 0.528\, x \qquad (11)$$

where c = toxic concentration (LC50), mg/ℓ,

x = number of carbon atoms in the alkyl chain.

The partition coefficients of these compounds also increase with increasing alkyl chain length and equation (11) could very likely be transformed into equation (2).

According to Wildish (1974), log(96hLC50) or log(96hLC0) is a linear function of the number of ethylene oxide units in polyoxyethylene esters, ethers, and amines. The toxicity decreases with increasing length of the polyoxyethylene chain. It should be noted that the experiments were carried out with technical preparations so that the numbers of ethylene oxide units are only mean values, and the toxicities have not been expressed on a molar basis.

Among industrial chemicals, phthalates offer an opportunity to examine their toxicity in relation to partition coefficient and water solubility. The data of Sugawara (1974) show that within the polymer-homologous series methyl-, ethyl-, butyl-, hexyl-, and octyl, the toxicity to shrimp eggs reaches a maximum for dibutyl phthalate.

CORRELATIONS OF CHEMICAL OR BIOCHEMICAL PROPERTIES AND BIOLOGICAL ACTIVITY

Correlations of easily determinable chemical or biochemical properties of compounds with their biological activity may also be very useful for the assessment of environmental properties of chemicals. In comparison with the above-mentioned correlations, little work has been done in this area by aquatic toxicologists.

Correlations between the inhibition of acetylcholinesterase and insect toxicity of organophosphorus pesticides can be found frequently in the literature, but have not been used extensively in the case of aquatic fauna.

Bond and Fuller (1971) described a correlation between the sulfhydryl-group blocking activity and acute toxicity of alkylvinyl sulfones to freshwater snails, and Gafa (1974) obtained correlations between surface tension of anionic surfactants and median lethal concentration to *Carassius auratus*.

SUGGESTED GROUPS OF COMPOUNDS FOR STRUCTURE-ACTIVITY CORRELATIONS

Substituted salicylanilides are probably the largest group of related compounds, examined for fish toxicity (Walker et al. 1966, Marking and Willford 1970). The data have not been quantitatively evaluated in terms of free-energy related constants.

The commercial availability of numerous chlorobiphenyls makes them an attractive group for structure-activity correlations. Fish metabolize most chlorobiphenyls only very slowly, if at all, and it may not be possible to obtain a correlation between structure and metabolism.

Aromatic and aliphatic hydrocarbons are another interesting group of compounds for structure-activity correlations. In this case fish are

suitable for metabolic, and shellfish for accumulation studies.

It is logical to expect that above a certain molecular weight compounds will not be taken up by aquatic animals. The limiting molecular weight may be approximately 600, as indicated by our data on the uptake of PCB and chlorinated paraffins by juvenile Atlantic salmon (Zitko 1974). It would be interesting to see whether any generalizations on uptake — molecular weight relationships can be made.

Compounds of low water solubility are present in the aquatic environment mostly adsorbed on suspended matter and, depending on the strength of adsorption, may or may not be available to aquatic fauna. For example, PCB and very likely DDT and related compounds are taken up by fish from suspended solids (Zitko 1974). This area of research deserves more attention. Some useful generalizations may be obtained.

CONCLUSIONS

The presented review of quantitative structure (properties)-activity relationships in aquatic toxicology indicates that these relationships are a useful tool for the assessment of environmental properties of organic compounds, and that this direction of research deserves more attention. A considerable progress could be achieved by determining structure-activity relationships for some typical non-target aquatic species already during the development of new pesticides and chemicals, expected to reach the environment from industrial sources. Sets of structural constants should be standardized as much as possible to make data from different laboratories easily comparable.

Generalizations of structure-activity relationships are complicated by the great diversity of aquatic fauna and its widely varied metabolic capabilities and responses to chemicals.

The relationships will answer many questions about environmental properties of organic compounds, but their major limitation is the choice of appropriate types of biological activity.

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SURFACTANT STRUCTURE AND AQUATIC TOXICITY

CHAPTER 2

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ABSTRACT

Studies of readily metabolized 14C surfactant compounds make it necessary to question the pertinence of partition coefficient data as a meaningful predictive tool of bioaccumulation and aquatic toxicity. Water/Octanol partition coefficients were estimated for surfactant components and the results compared to acute toxicity and bioaccumulation of Daphnia and fathead minnows. Additional questions are raised concerning the methods used to measure the partition coefficients. As far as acute toxicity is concerned, a good relative correlation between it and the measured partition coefficients was shown for the components of surfactants. However, we are not certain about the absoluteness of correlations.

INTRODUCTION

There is a current need to develop methods of estimating the relationships between chemical structure and aquatic toxicological properties of compounds reaching the environment. The octanol/water partition coefficient is a physical measurement that has been used for many years by pharmacologists to estimate the biological activity of compounds. In recent years, aquatic toxicologists have attempted to correlate the bioaccumulation potential of compounds with partition coefficients (Neely, et al., 1974). To date, no such studies have been reported on surfactants.

Linear alkyl benzene sulfonate (LAS) is a major anionic surfactant in commercial use (Figure 1). It is readily and completely biodegradable by ordinary environmental bacteria. Therefore, aquatic organisms are rarely exposed to intact LAS. The many studies that have been conducted on biodegradation (Swisher, 1970) and the aquatic toxicity (Hirsch, 1963; Swisher, et al., 1964; Borstlap, 1967; Marchetti, 1965; Divo, 1974) lead to a general conclusion that biological activity, microbial through vertebrate, is related to alkyl chain length and phenyl isomer position. Longer chains and terminal isomers are the more toxic but are also the first components to biodegrade. The biodegradation process in LAS

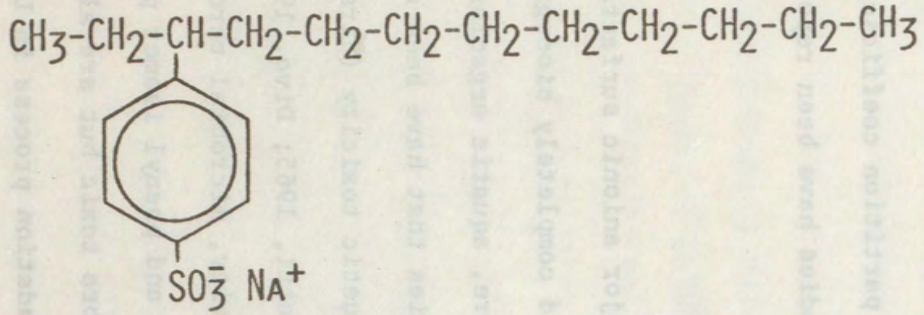

FIGURE 1. TYPICAL LAS MOLECULE, 3-PHENYLDODECANE SULFONATE. (COMMERCIAL LAS IS A MIXTURE OF 15-25 SUCH ISOMERS AND HOMOLOGS WITH ALKYL CHAIN LENGTHS RANGING FROM 10 TO 14 CARBONS, PHENYL GROUPS DISTRIBUTED APPROXIMATELY EVENLY AT ALL POSITIONS ALONG THE CHAINS EXCEPT VERY FEW AT THE TWO ENDS, AND SULFONATE GROUP PREDOMINANTLY IN THE PARA POSITION).

proceeds in short steps giving a series of carboxylated molecules, each one of which is one step nearer to complete degradation. As a result of these changes in the LAS molecule, there is a concurrent increase in hydrophilic properties and a loss of surfactancy properties and of toxicity to aquatic organisms. Swisher, et al. (1964) demonstrated that bluegill could survive in effluents from laboratory continuous flow activated sludge units being fed 100 mg/l of LAS, samples which in the intact form had LC_{50}'s in the range of 0.6-3.0 mg/liter. A model carboxylated intermediate, sulfophenylundacanoic acid disodium salt, had a 24 hr. LC_{50} value of 120 mg/l. The role of the preferentially faster rate of biodegradation of the more toxic longer chain lengths and terminally positioned isomers was demonstrated by Divo (1974) with significant reductions in toxicity as the percent biodegradation increased.

The purpose of the present study was to determine (1) the factors influencing measurement of the octanol/water partition coefficients of surfactants and (2) the relationship of surfactant partition coefficient to bioaccumulation and acute toxicity. The surfactants studied were a series of LAS individual homologs with alkyl chain lengths from 9 to 15 carbon atoms, representative commercial LAS blends, and a carboxylated LAS simulating a degradation metabolite.

Surfactants are unusual molecules because their structural characteristics combine both hydrophilic and hydrophobic-lipophilic groups in the same

molecule. This makes them diviate from the laws of ideal solutions. It can be expected that partition coefficients of surfactants may vary by orders of magnitude depending on such factors as solute concentration, solvent ratio, water hardness, pH, and operating procedures. These same factors may also come into play in the measurement of bioaccumulation/aquatic toxicity. After a finite time of exposure the organism will come into some sort of equilibrium with the aqueous phase. On the one hand, if the solute is conserved (i.e., is not metabolized by the organism) its accumulation in various tissues would presumably be governed by passage through membranes for the rate and by the appropriate lipid/water partition coefficient for the extent. In turn, the ultimate toxicity would be determined by the extent of accumulation and the intrinsic toxicity of the compound. On the other hand, if the compound is metabolized by the organism, the partition coefficient or indeed the intrinsic toxicity of the intact original compound may become more or less irrelevant. This would be particularly true if the compound were completely metabolized to carbon dioxide and water.

MATERIALS AND METHODS

Materials

LAS samples were prepared by sulfonation of the corresponding alkylbenzenes in the usual manner, neutralizing the sulfonic-sulfuric acid mixture in 80% isopropyl alcohol, filtering out the precipitated sodium sulfate and drying the filtrate to give the LAS in about 99% purity, the remaining 1% being mainly sodium sulfate and moisture. The ^{14}C tagged LAS samples were

prepared by alkylation of uniformly labeled benzene with the appropriate olefins. The sulfonates were neutralized in water instead of isopropyl alcohol, and hence were accompanied by about 10% of sodium sulfate. Compositions of the samples are summarized in Table I.

Sulfophenylundecanoic Acid Disodium Salt was obtained in a similar manner by sulfonation of phenylundecanoic acid (Eastman 5352) (Swisher, et al., 1964).

Two Non-Surfactant ^{14}C compounds, a water soluble chelant and a water insoluble biocide, were also studies to compare these extremes with the surfactants.

Analytical

In Solutions

LAS was determined by the methylene blue procedure and by ^{14}C counting as well in the tagged samples.

Methylene blue analyses were by the Hellige modification (Swisher, et al., 1964) in which a 50 ml sample is shaken with 15 ml Standard Methods reagent plus 10 ml chloroform and the blue color is compared with glass standards ranging from 0 to 2 ppm. Uncertainty of the comparison is about ± 0.05 ppm over most of the scale, ± 0.1 at the upper end. Samples over about 1.5 ppm are diluted down to approximately 1 ppm before analysis. At the lower end of the scale, levels of 0.05 ppm can be distinguished from zero and 0.1

Table I

Composition of LAS Samples

Sample	A	B	C	D	E	F	G	H*	I*	J*	K*
Ave. Chain Length	9	10	11	12	13	14	15	12	13	11.6	13.1
Chain Length, %											
9	99	1									
10		99	1							6	
11			99	2						37	1
12				98	1			100	6	53	10
13					99	1			94	4	60
14						99					29
15							100				
2-Phenyl Isomer, %	28	24	18	14	19	17	15	17	17	18	12

*Uniformly labeled with ^{14}C in the ring.

with reasonable confidence. Analysis of the octanol layer was accomplished by using a sample containing up to about 50 µg of LAS, but not more than 5 ml of octanol, along with enough chloroform to make the total volume 10 ml, adding 50 ml of deionized water and 15 ml methylene blue reagent and proceeding as above.

Radiocarbon counts were made by adding an appropriate amount of sample (up to 5 ml of aqueous or octanol layer) to 15 ml of Packard Instagel in a glass vial and counting in a Nuclear Chicago Isocap 300 liquid scintillation counter.

In Tissues

Duplicate samples of Daphnia and fathead minnow tissues and organs were dried, weighed and transferred to planchets. Radiocarbon content was determined using a Peterson type burning-CO_2 collection apparatus (Peterson, 1969). Counting was as above.

Determination of intact LAS in the tissues was accomplished by desulfonation-gas chromatography (Swisher, 1963; Sullivan, Swisher, 1969). The dried tissue sample was extracted by boiling with several successive portions of methanol, filtering through paper. About 90-95% of the ^{14}C appeared in the methanol. For desulfonation, an amount of methanol corresponding to 100 µg of the original LAS was spiked with 10 µg each of 1-sulfophenyl-decane, -undecane and -dodecane as internal standard, evaporated to dryness

and desulfonated at 200°C in boiling phosphoric acid for 1 1/2 hours, the resulting alkylbenzenes being continuously extracted from the overhead reflux into 1 ml hexane which was finally concentrated to a volume of 50 λ. Standard runs were also made starting with 100 µg each of the original tagged LAS plus 10 µg each of the three internal standards. Recovery of ^{14}C in the desulfonation was generally 50-100%.

Gas chromatography was done in a Hewlett-Packard 5710A instrument with a 150' X 0.02" column, DC 550 substrate, 170°C, flame ionization detector, 1 λ sample of hexane concentrate, split ratio 4:1. Amount of intact LAS in the tissue was estimated from the individual alkylbenzene peak heights in comparison with those from the standard run on the initial LAS, with appropriate reference to the alkylbenzene peaks from the three internal standards. Interferences arose from the lipids and other natural components of the tissues which gave volatile products during the desulfonation treatment, in amounts several orders of magnitude greater than the alkylbenzenes, so that the precision of the results is poor except in the case of the gall bladders, where the interferences were much lower.

Partition Coefficients

In earlier runs separatory funnels (250-500 ml) were shaken gently by hand for 2 minutes and settled overnight. The resulting aqueous layer was slightly turbid, while the octanol layer usually remained highly emulsified; centrifugation at 1000 g for 10-20 minutes did not improve the clarity significantly.

In the preferred procedure equilibrations were accomplished in Erlenmeyer flasks (125-1000 ml) on a rotary shaker at 100 rpm for 24 hours (8 hours would probably have been sufficient). Both phases remained clear except for a few cases in which the lower layer developed a slight turbidity.

The test materials were first dissolved in water at the desired concentration (usually 1 or 10 mg/liter) in the separatory funnel or Erlenmeyer flask, a sample was withdrawn for analysis, the desired volume of 1-octanol (Fisher A-402, used without further purification) was added. After equilibration, samples of the lower and (if not emulsified) upper layers were taken with precautions to avoid intermixing during withdrawal. In the subsequent calculations the volumes of the two phases after equilibration were assumed to be the same as the initial volumes of water and octanol, without correction for possible minor changes due to mutual solubility.

The amount of solute in the upper layer was calculated as the difference between the analyzed amounts in the lower layer initially and after equilibration, and the concentration in the upper layer by dividing by the volume of upper layer. A second value for the concentration in the upper layer was available in those cases wherein the upper layer could be analyzed directly. In general, these two values agreed reasonably well. Partition coefficients were calculated as the ratio of concentration in the octanol phase to that in the water phase. All of the LAS samples were mixtures containing from 4 up to 10-15 components, some with widely differing partition

coefficients, but no attempt was made to calculate or correct for the differences in composition which undoubtedly took place upon partition. A single, average partition coefficient was simply calculated for each run made.

The volumes of water and octanol were chosen to be consistent with the limitations of analytical sensitivity and precision. Starting at 1 mg/liter the initial aqueous solution was usually 200 ml, giving 50 ml for initial analysis and leaving 150 ml for partition. Starting at 10 mg/liter, initial volumes as low as 50 ml were made up, 10 being reserved for analysis and 40 going into partition. The water:octanol ratios were as low as 2:1 with the shorter chain homologs to allow sufficient removal of LAS from the aqueous phase to make an analytically significant difference in concentration. With the longer homologs and in hard water, ratios of 100:1 or more were required if sufficient LAS was to be left in the aqueous phase for meaningful analysis. In all cases the volume of flask was chosen so that it would be about half full.

Acute Toxicity Tests

All acute toxicity tests were conducted with Daphnia magna following the procedures of the Committee on Methods for Toxicity Tests with Aquatic Organisms (Stephan, C., 1974, Personal Communications). Ten first instar daphnids were placed in 250 ml beakers containing 200 ml of well water with a $CaCO_3$ hardness of either 50 or 250 mg/l. Test compounds were added

before daphnids. Each experiment consisted of 5 to 6 concentrations of the compound with 3 replicates for each concentration. The duration of the test was 48 hours with mortalities recorded at 24 and 48 hours. No food was provided. LC_{50} values and 95% confidence limits were calculated using a logit transformation computer program following the method of Litchfield-Wilcoxon.

Bioaccumulation Tests

Bioaccumulation studies were conducted with Daphnia magna and fathead minnows (Pimephales promelas) in well water of 250 mg/1 $CaCO_3$ hardness. All tests were continuous flow with 3 to 4 aquaria volumes exchanged each day. Daphnia and minnow tests were conducted in glass 15-liter and 60-liter aquaria, respectively. Test compounds were metered into aquaria using a peristalic pump. Daphnia were fed 200-300 mg of a trout-chow alfalfa suspension each day. Fathead minnows received a daily ration of trout-chow equal to ∿2% of their 1 g body weight. The duration of bioaccumulation tests were 21 to 28 days. Daphnia and fish were usually removed on days 1, 3, 7, 11, 14, 21, and 28. Approximately 100 to 20 daphnids, depending on their size, were used for each estimate of accumulated ^{14}C tag. Four fish were dissected on each sampling date with 2 fish per data point and 2 data points per mean estimate.

RESULTS AND DISCUSSION

Partition Coefficients

As indicated earlier, surfactants are unusual molecules and measuring partition coefficients was drastically influenced by a number of factors. Figure 2 shows the partition coefficients determined for each LAS chain length. As expected, the partition coefficients increased with increasing chain length. However, in deionized water the incremental increase of one carbon in the chain did not yield the 1/2 log increase in the partition coefficient noted by Hansch (1972), instead it was about 1/3 of a log. The effect of hardness was obvious with a 10 to 100 fold increase in partition coefficients determined in 250 mg/l hardness water as compared to those determined in deionized water. Here the incremental increase was about 1/2 log per carbon. The relationships between and effects of hardness, pH and quantity of octanol are not entirely understood at this time. Indeed, in many cases the results were not as reproducible as we would like. Further studies are being conducted.

Figure 2 also shows that the concentration of the surfactant can affect the partition coefficients, even when all other factors were held approximately constant. A concentration of 1 mg/l LAS yielded partition coefficients about 10 times greater than those starting at 10 mg/liter.

Figure 3 shows that the sulfophenylundecanoic acid disodium salt partition coefficient was relatively unaffected by water hardness. The partition coefficient of this simulated metabolite was approxiamtely 1/10 of the C_{11} LAS partition coefficient.

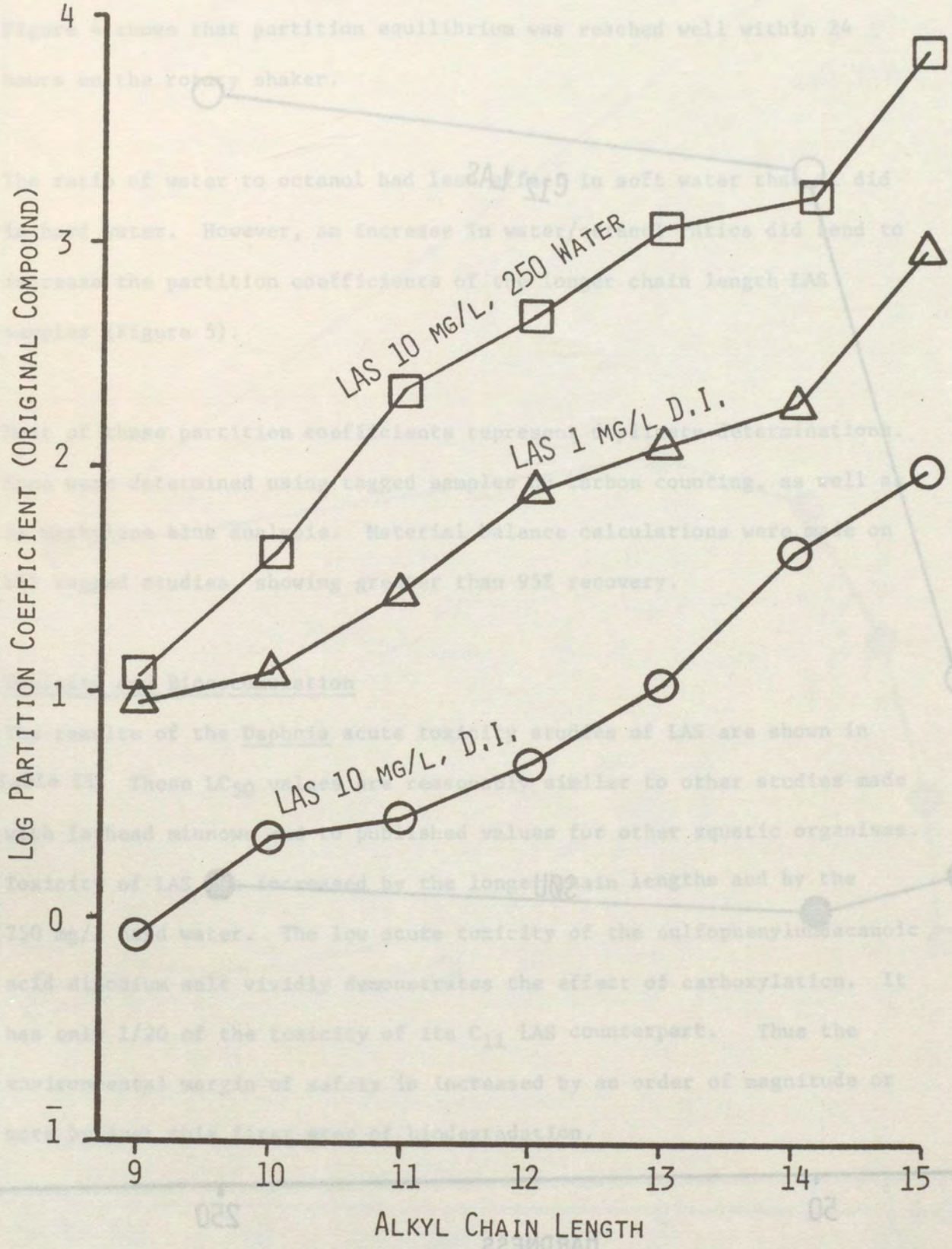

Figure 2. Comparison of Partition Coefficients for Each Alkyl Chain Length 9 through 15 in Deionized Water and Well Water of 250 mg/l and at LAS Concentrations of 1 and 10 mg/l.

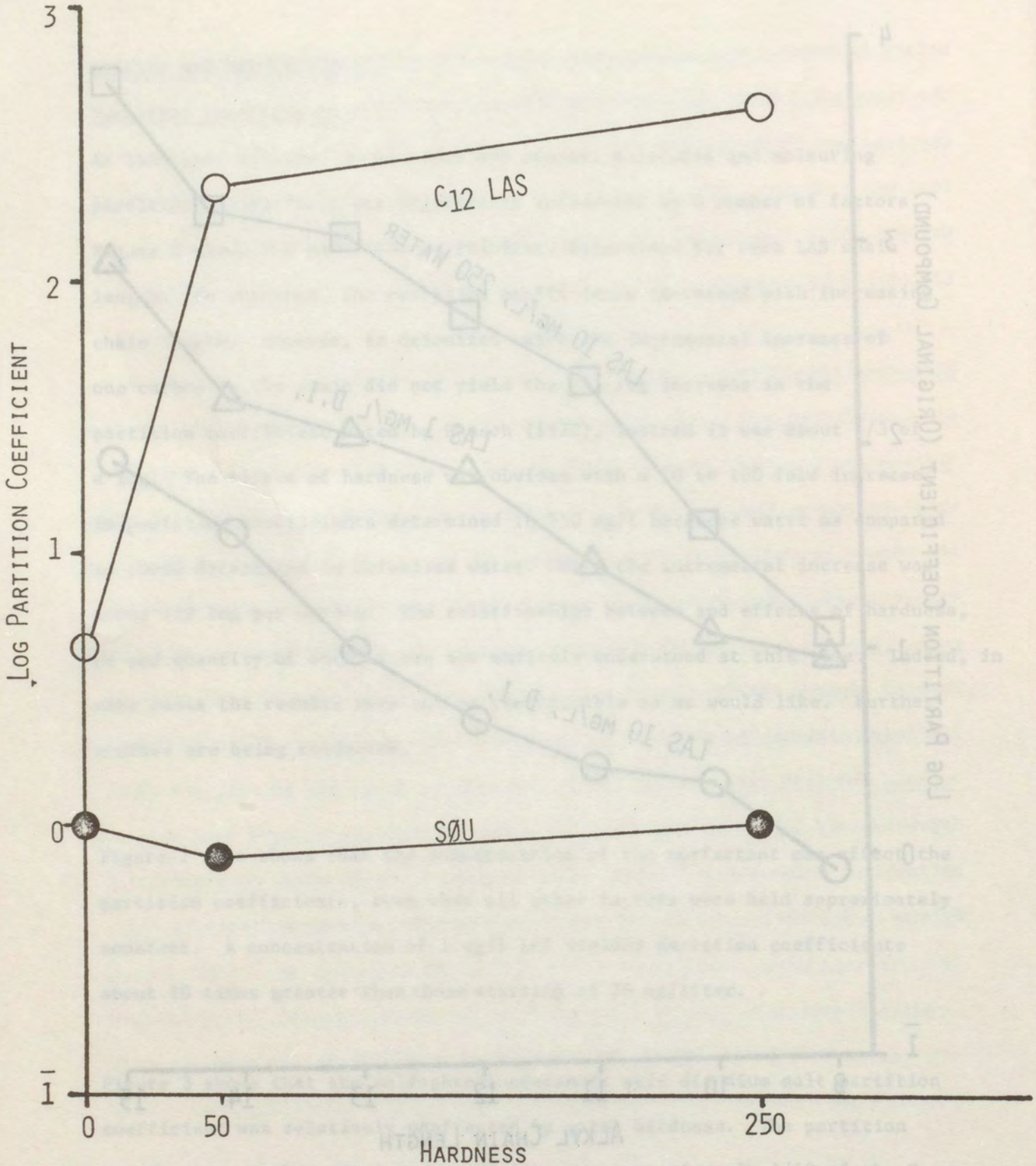

FIGURE 3. COMPARISON OF THE EFFECT OF WATER HARDNESS ON THE PARTITION COEFFICIENT OF C_{12} LAS AND A MODEL CARBOXYLATED DEGRADATION METABOLITE (SULFOPHENYLUNDECANOIC ACID DISODIUM SALT), BOTH AT 10 MG/L.

Figure 4 shows that partition equilibrium was reached well within 24 hours on the rotary shaker.

The ratio of water to octanol had less effect in soft water than it did in hard water. However, an increase in water/octanol ratios did tend to increase the partition coefficients of the longer chain length LAS samples (Figure 5).

Most of these partition coefficients represent duplicate determinations. Some were determined using tagged samples by carbon counting, as well as by methylene blue analysis. Material balance calculations were made on all tagged studies, showing greater than 95% recovery.

Toxicity and Bioaccumulation

The results of the Daphnia acute toxicity studies of LAS are shown in Table II. These LC_{50} values are reasonably similar to other studies made with fathead minnows and to published values for other aquatic organisms. Toxicity of LAS was increased by the longer chain lengths and by the 250 mg/l hard water. The low acute toxicity of the sulfophenylundecanoic acid disodium salt vividly demonstrates the effect of carboxylation. It has only 1/20 of the toxicity of its C_{11} LAS counterpart. Thus the environmental margin of safety is increased by an order of magnitude or more by just this first step of biodegradation.

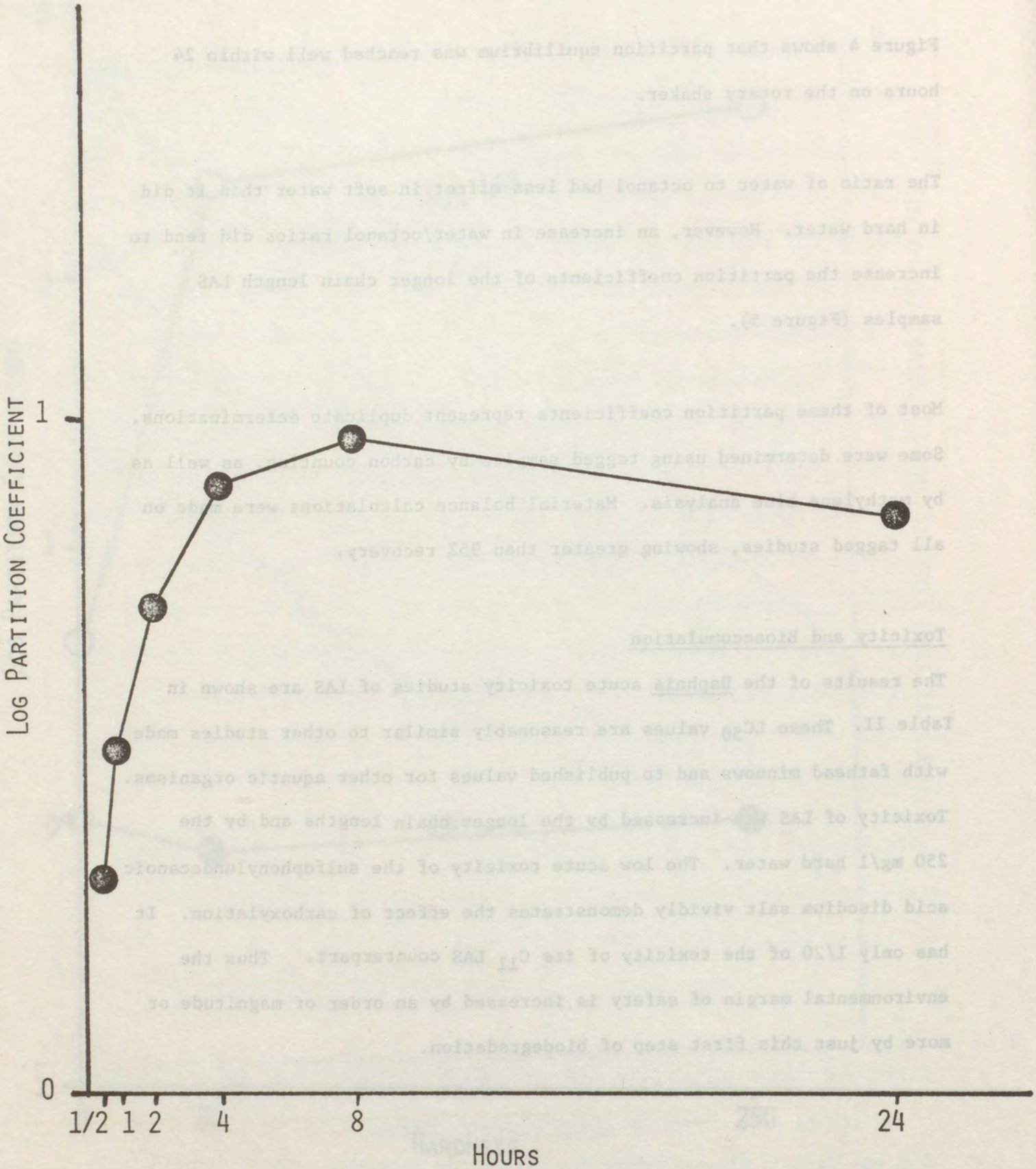

FIGURE 4. EFFECT OF MIXING TIME ON APPARENT PARTITION COEFFICIENTS OF C_{12} LAS AT 10 MG/L IN DEIONIZED WATER

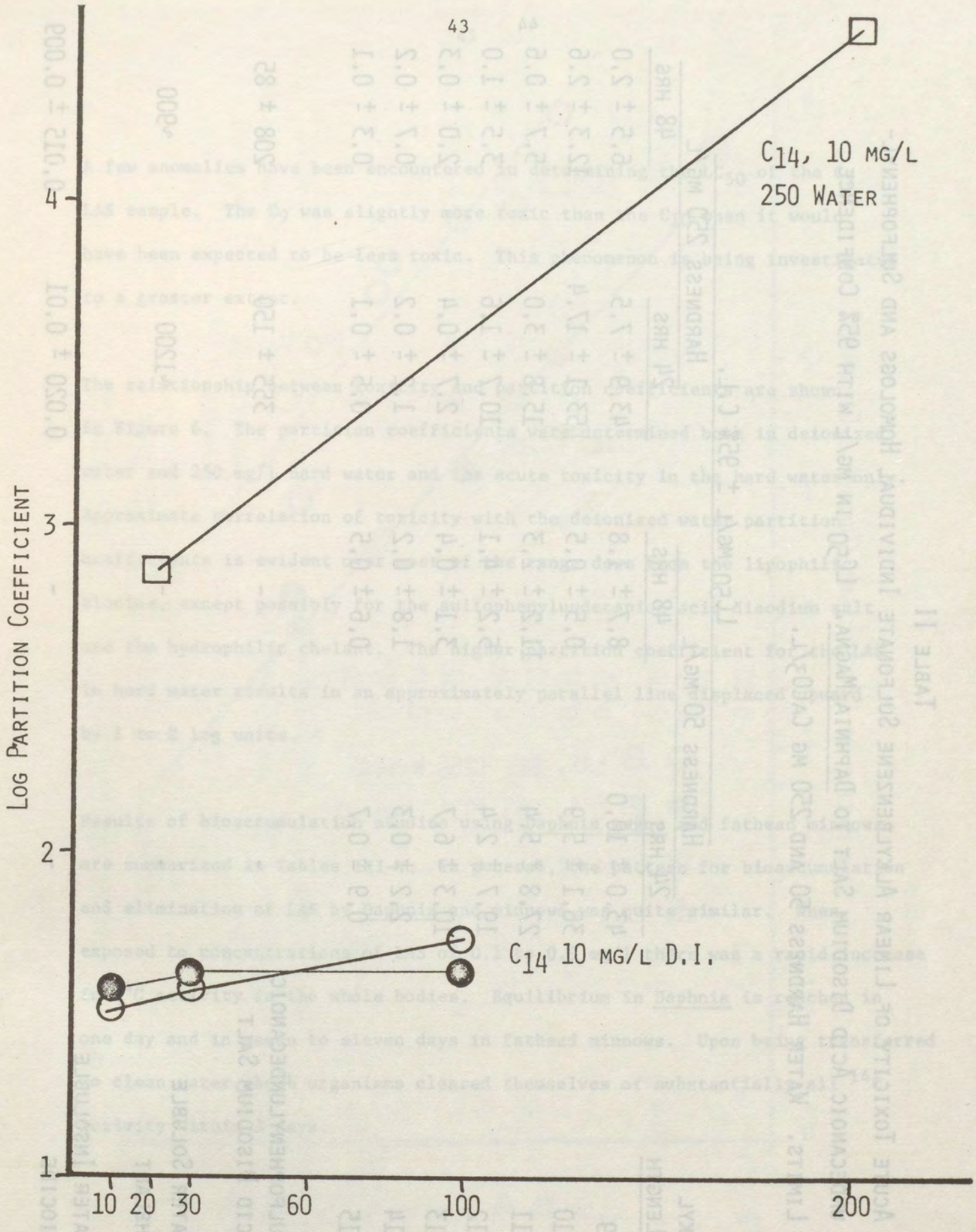

FIGURE 5. INFLUENCE OF WATER/OCTANOL RATIOS ON PARTITION COEFFICIENT OF LAS SAMPLES

Table II

Acute Toxicity of Linear Alkylbenzene Sulfonate Individual Homologs and Sulfophenyl-undecanoic Acid Disodium Salt to Daphnia Magna. LC_{50} in mg/L with 95% Confidence Limits. Water Hardness 50 and 250 mg $CaCO_3$/L.

LAS Alkyl Chain Length	LC_{50} mg/L ± 95% C.L.			
	Hardness 50 mg/L		Hardness 250 mg/L	
	24 hrs	48 hrs	24 hrs	48 hrs
C_9	43.0 ± 10.0	8.7 ± 1.8	43.9 ± 7.5	6.5 ± 2.0
C_{10}	36.1 ± 5.9	0.5 ± 0.5	53.1 ± 17.4	12.3 ± 2.6
C_{11}	27.8 ± 5.4	11.2 ± 2.3	15.8 ± 3.0	5.7 ± 0.6
C_{12}	19.7 ± 2.4	5.2 ± 4.1	10.7 ± 1.6	3.5 ± 1.0
C_{13}	10.3 ± 6.7	3.1 ± 0.4	2.7 ± 0.4	2.0 ± 0.3
C_{14}	2.2 ± 0.3	1.8 ± 0.2	1.2 ± 0.2	0.7 ± 0.2
C_{15}	0.9 ± 0.7	0.6 ± 0.5	0.5 ± 0.1	0.3 ± 0.1
Sulfophenylundecanoic Acid Disodium Salt	-	-	355 ± 150	208 ± 85
Water Soluble Chelant	-	-	~1200	~900
Water Insoluble Biocide	-	-	0.020 ± 0.01	0.015 ± 0.009

A few anomalies have been encountered in determining the LC_{50} of the C_9 LAS sample. The C_9 was slightly more toxic than the C_{10} when it would have been expected to be less toxic. This phenomenon is being investigated to a greater extent.

The relationship between toxicity and partition coefficients are shown in Figure 6. The partition coefficients were determined both in deionized water and 250 mg/l hard water and the acute toxicity in the hard water only. Approximate correlation of toxicity with the deionized water partition coefficients is evident over most of the range down from the lipophilic biocide, except possibly for the sulfophenylundecanioc acid disodium salt and the hydrophilic chelant. The higher partition coefficient for the LAS in hard water results in an approximately parallel line displaced upward by 1 to 2 log units.

Results of bioaccumulation studies using Daphnia magna and fathead minnows are summarized in Tables III-V. In general, the pattern for bioaccumulation and elimination of LAS by Daphnia and minnows was quite similar. When exposed to concentrations of LAS of 0.1 to 0.9 mg/l there was a rapid increase in ^{14}C activity in the whole bodies. Equilibrium in Daphnia is reached in one day and in seven to eleven days in fathead minnows. Upon being transferred to clean water, both organisms cleared themselves of substantially all ^{14}C activity within 3 days.

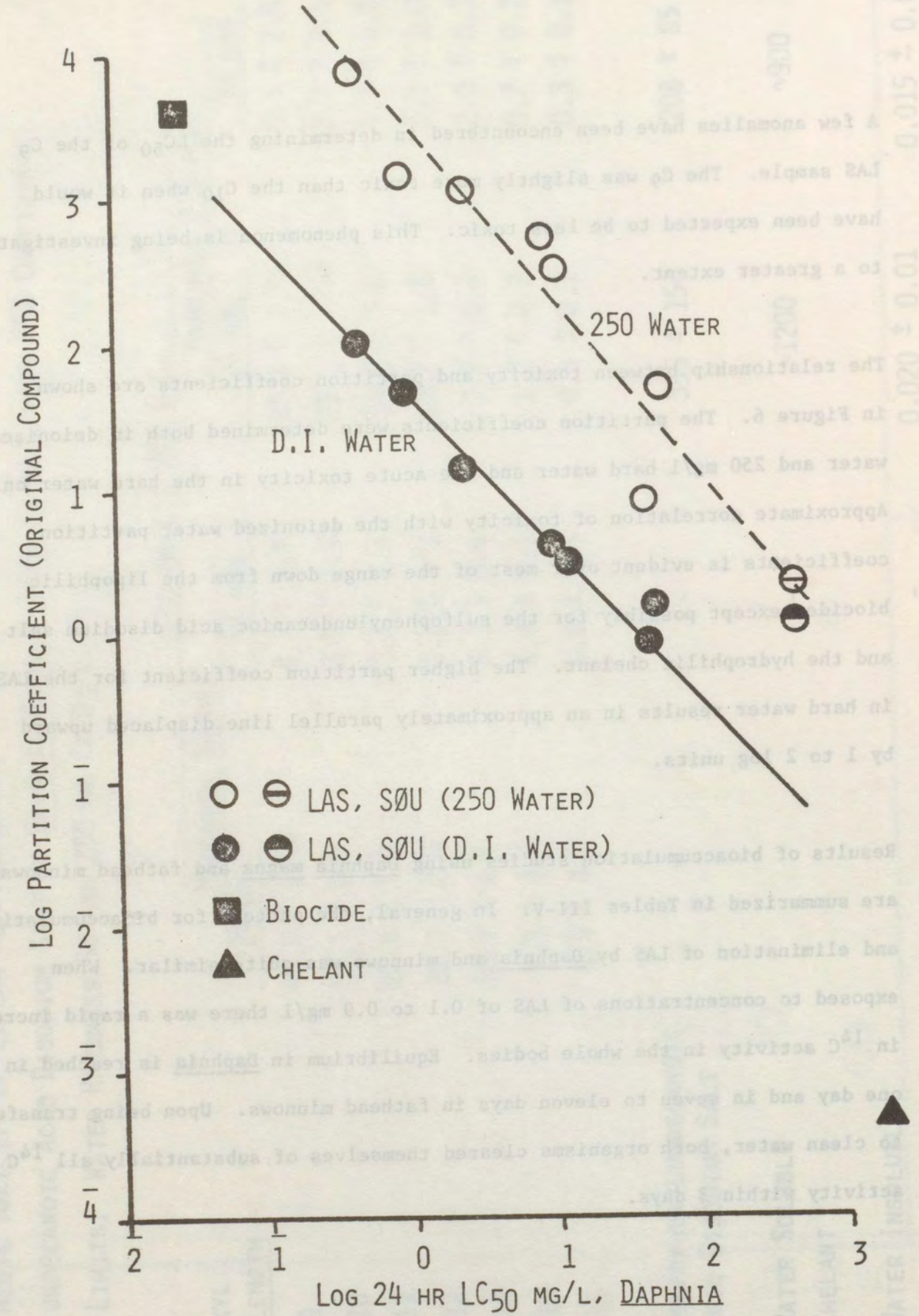

Figure 6. Relationship Between Partition Coefficient and Acute Toxicity of LAS Pure Homologs, C_9-C_{15}, Model Degradation Intermediate (Sulfophenylundecanoic Acid Disodium Salt (SØU), Biocide and Chelant.

Table III

Apparent Bioaccumulation of LAS Pure Homologs and Blends in *Daphnia Magna* at 14 Days

^{14}C LAS Sample	Log Partition Coefficient	Concentration Tested mg/L	Apparent Uptake At Equilibrium µg/g (wet)	Apparent Concentration Factor
C_{12} Pure (H)	0.93	0.07	0.6	8
		0.16	9.2	58
		0.44	39.3	89
		0.44	51.2	116
C_{13} Pure (I)	1.29	0.09	12.6	140
		0.09	13.0	144
		0.41	92.3	225
		0.41	104.5	255
$C_{11.6}$ Blend (J)	0.60	0.90	43.7	49
		0.90	45.7	51
$C_{13.1}$ Blend (K)	1.59	0.90	485.3	539
		0.90	767.7	853

Table IV

Apparent Bioaccumulation of LAS Pure Homologs C_{12} and C_{13} in Fathead Minnows at 14 Days; Whole Fish, Muscle Tissue and Gall Bladders

^{14}C LAS Sample	Log Partition Coefficient	Tissue	Concentration Tested mg/L	Apparent Uptake At Equilibrium µg/g (wet)	Apparent Concentration Factor
C_{12} (H)	0.93	Whole Fish	0.100	17.3	173
		Muscle	0.100	0.4	4
		Gall Bladder	0.100	1372.2	13,700
C_{12} (H)	0.93	Whole Fish	0.135	33.3	245
		Muscle	0.135	0.4	3
		Gall Bladder	0.135	1012.0	7,500
C_{13} (I)	1.29	Whole Fish	0.100	38.5	385
		Muscle	0.100	1.3	13
		Gall Bladder	0.100	3064.7	30,647
C_{13} (I)	1.29	Whole Fish	0.114	33.5	293
		Muscle	0.114	4.0	35
		Gall Bladder	0.114	1165.0	10,200

TABLE V

BIOACCUMULATION IN DAPHNIA MAGNA OF 2 COMPOUNDS WIDELY DIVERGENT IN THEIR WATER SOLUBILITY PROPERTIES

COMPOUND	LOG PARTITION COEFFICIENT	CONCENTRATION TESTED µG/L	UPTAKE AT EQUILIBRIUM µG/G (WET)	CONCENTRATION FACTOR
WATER SOLUBLE CHELANT	-3.59	1.0	0.02	20
		100.0	0.8	8
WATER INSOLUBLE BIOCIDE	3.72	0.5	2.0	4000

Figure 7 presents the relationship between partition coefficients and the bioaccumulation factor for four LAS samples, using the data from Tables III-V. The two extreme data points, one the hydrophilic compound and the other the lipophilic compound, indicate an apparent "S" shaped curve. As was the case with acute toxicity, a good relationship is indicated for the LAS and Daphnia through the middle portion of the curve. Intact whole fathead minnow data also fall in that range. However, the muscle and gall bladder data are widely divergent. The reason is that the LAS is easily and rapidly metabolized by fish, probably irrespective of the partition coefficient or chain length. By extraction and desulfonation-GC analysis we have determined that the ^{14}C activity in the various organisms and tissues is not entirely LAS. Indeed the gall bladders contained essentially no LAS, the ^{14}C found being very probably in the form of short chain carboxylates with the aromatic ring still intact. The tag recovered from muscle tissue was 50 to 70% LAS. In the other organs and tissues only 20 to 50% of the total ^{14}C was intact LAS.

These results suggest certain limitations in the applicability of partition coefficient data to prediction of bioaccumulation and certain precautions to be observed in the use of tagged compounds for such studies. Although the partition coefficient measured for the original compound may govern its input rate into the organism, the clearance of the metabolites will depend on their own partition coefficients, which may be greatly different. Hence the steady state level of tagged materials is not dependent solely on the

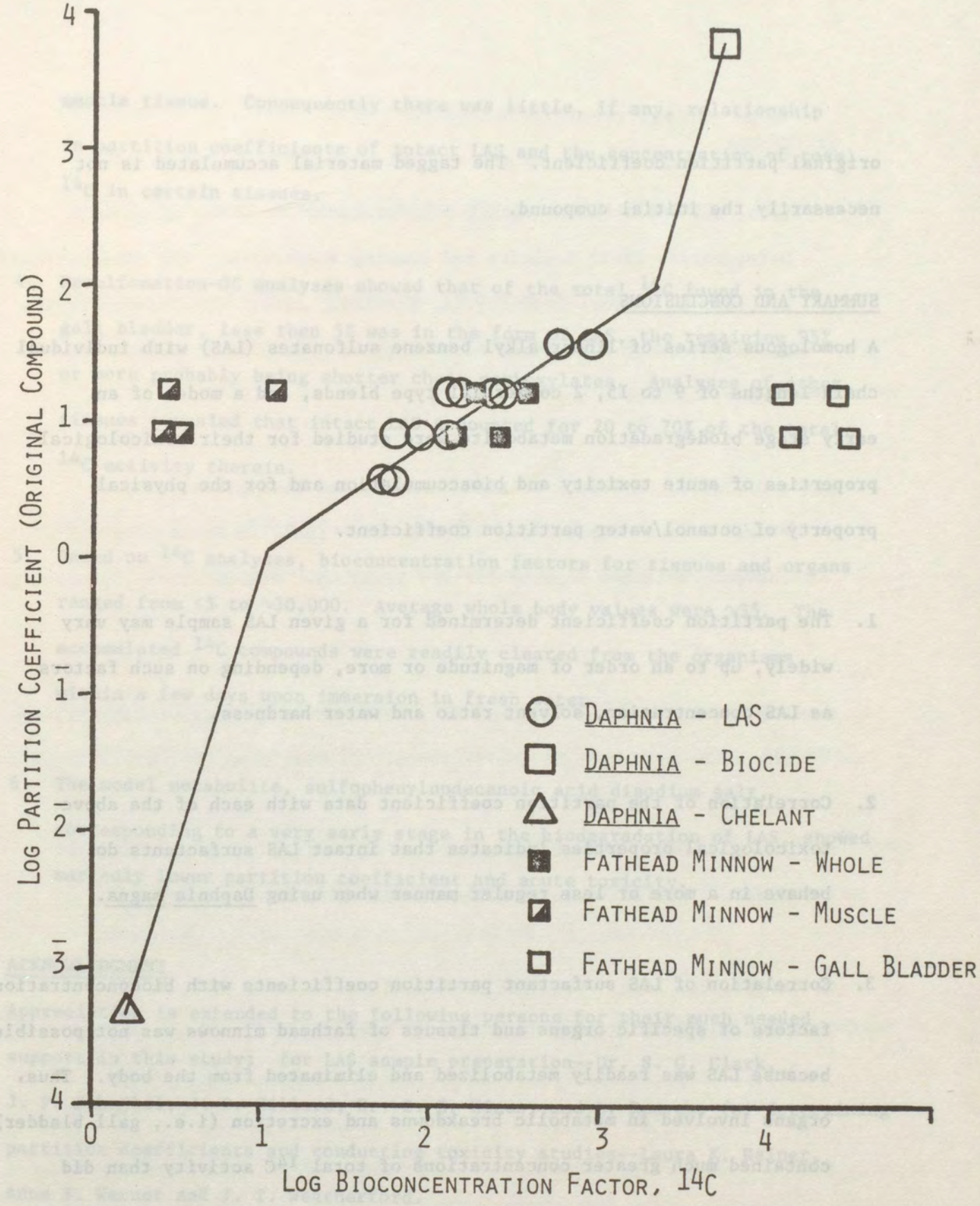

Figure 7. Relationship Between Partition Coefficient and Bioconcentration factor. Referred to the Total ^{14}C Content of the Tissues, the Fathead Muscle Contained 30-70% of Non-LAS, the Whole Minnow 70-80% and the Gall Bladder 97% or More.

original partition coefficient. The tagged material accumulated is not necessarily the initial compound.

SUMMARY AND CONCLUSIONS

A homologous series of linear alkyl benzene sulfonates (LAS) with individual chain lengths of 9 to 15, 2 commercial type blends, and a model of an early stage biodegradation metabolite were studied for their toxicological properties of acute toxicity and bioaccumulation and for the physical property of octanol/water partition coefficient.

1. The partition coefficient determined for a given LAS sample may vary widely, up to an order of magnitude or more, depending on such factors as LAS concentration, solvent ratio and water hardness.

2. Correlation of the partition coefficient data with each of the above toxicological properties indicates that intact LAS surfactants do behave in a more or less regular manner when using Daphnia magna.

3. Correlation of LAS surfactant partition coefficients with bioconcentration factors of specific organs and tissues of fathead minnows was not possible because LAS was readily metabolized and eliminated from the body. Thus, organs involved in metabolic breakdowns and excretion (i.e., gall bladder) contained much greater concentrations of total ^{14}C activity than did

muscle tissue. Consequently there was little, if any, relationship in partition coefficients of intact LAS and the concentration of total ^{14}C in certain tissues.

4. Desulfonation-GC analyses showed that of the total ^{14}C found in the gall bladder, less than 5% was in the form of LAS, the remaining 95% or more probably being shorter chain carboxylates. Analyses of other tissues revealed that intact LAS accounted for 20 to 70% of the total ^{14}C activity therein.

5. Based on ^{14}C analyses, bioconcentration factors for tissues and organs ranged from <5 to ∿30,000. Average whole body values were ∿35. The accumulated ^{14}C compounds were readily cleared from the organisms within a few days upon immersion in fresh water.

6. The model metabolite, sulfophenylundecanoic acid disodium salt, corresponding to a very early stage in the biodegradation of LAS, showed markedly lower partition coefficient and acute toxicity.

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STRUCTURE-ACTIVITY RELATIONSHIPS APPLIED

CHAPTER 3

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ABSTRACT

A correlation showing the dependency of the observed biological activity (LC_{50}) of a series of phenols to the free energy related terms, π, F and R (field and resonance), has been observed for the freshwater invertebrate, Daphnia magna. A series of 14 phenols was investigated with the best correlation obtained with F and R considered to be additive terms (Eqn. 1). Moreover, the dominant parameter in this study was found to be the partition coefficient (Eqn. 2).

Log 1/c = 0.500π plus 0.453F plus 0.63R plus 3.731

r = 0.978 (Eqn 1)

Log 1/c = 0.527π plus 3.796 r = 0.831 (Eqn 2)

The implications of these results for environmental problems associated with toxicity and bioaccumulation has led to the development of a method using a permanently bonded long chain alkyl packing in a high pressure liquid chromatographic system and subsequently relating the capacity factor k′ [$k' = (t_s - t_o)/t_o$, i.e., the net retention time relative to the nonadsorbed time] to the partition coefficient $P_{o/w}$.

Part I

Introduction

In the course of investigating the environmental impact of the products derived from the aqueous chlorination and ozonation of organic compounds known to exist in waste effluents, it became desirable to have a rapid and reliable bioassay for determining those compounds that possess high toxicity. Daphnia magna, which are in an intermediate relationship to other aquatic invertebrates, were chosen for the screening procedure. The choice was based on previous work[1-6] that has shown Daphnia magna to be relatively easy to rear and to manipulate, and to be responsive to added toxicants such as to be considered generally representative of aquatic invertebrates.

Phenols were studied because of their frequent appearance in effluents, the ability to obtain pure samples bearing systematic structural variations, and the significant amount of mechanistic and toxicity data available on phenol itself[7-12]. Moreover, phenols assume added significance when it is recognized that all of the compounds listed in Table 1 have the ability to incorporate chlorine over a wide range of pH and concentration[13].

CHART 2

COMPOUND	log P	log k′	calc. log P	π	κ	calc π
PHENOL SERIES						
-H	1.46	-.164	1.61	0.00	.000	0.15
3-OCH_3	1.56	-.213	1.52	0.10	-.049	0.06
4-NO_2	1.91	-.100	1.73	0.45	.064	0.27
4-CH_3	1.94	.025	1.97	0.48	.189	0.51
2-CH_3	1.95	.090	2.09	0.49	.254	0.63
2-Cl	2.15	.083	2.08	0.69	.247	0.62
2,6-CH_3	2.34	.241	2.38	0.88	.405	0.92
4-Cl	2.39	.233	2.37	0.93	.397	0.91
4-Br	2.59	.326	2.54	1.13	.490	1.08
ANILINE SERIES						
-H	0.90	-.216	0.95	0.00	.000	0.06
3-OCH_3	0.93	-.145	1.11	0.03	.071	0.21
4-OCH_3	0.95	-.321	0.72	0.05	-.105	-0.18
2-CH_3	1.32	-.004	1.43	0.42	.212	0.53
3-NO_2	1.37	.029	1.50	0.47	.245	0.60
4-CH_3	1.41	0.21	1.48	0.51	.237	0.58
3-CH_3	1.43	.004	1.45	0.53	.220	0.55
2-NO_2	1.79	.083	1.62	0.89	.299	0.72
4-Cl	1.83	.182	1.85	0.93	.398	0.95
2-Cl	1.92	.210	1.91	1.02	.426	1.01
4-Br	2.26	.262	2.02	1.36	.478	1.12
2,4-Cl	2.69	.585	2.75	1.79	.801	1.85

TABLE I

Toxicity of Phenols to Daphnia magna

Functions	$\Sigma\sigma$[a]	$\Sigma\pi$[b]	ΣF	ΣR	log 1/c[c] Calcd.	log 1/c[c] Observed	Δlog 1/c	c[b] (observed)
3-Methoxy	0.12	0.10	0.26	-0.51	3.574	3.480	-0.094	$3.31 \cdot 10^{-4}$
2-Methoxy	-0.27	0.22	0.26	-0.51	3.634	3.680	0.046	$2.09 \cdot 10^{-4}$
4-Methyl	-0.17	0.48	0.04	-0.13	3.907	3.709	-0.198	$1.95 \cdot 10^{-4}$
2-Methyl	-0.17	0.49	0.04	-0.13	3.912	3.835	-0.077	$1.46 \cdot 10^{-4}$
H	0.00	0.00	0.00	0.00	3.731	3.904	0.173	$1.02 \cdot 10^{-4}$
2,6-Dimethyl	-0.34	0.88	0.08	-0.26	4.042	4.036	-0.00	$9.20 \cdot 10^{-5}$
4-Nitro[d]	1.22	0.50	0.67	0.16	4.387	4.219	-0.168	$6.04 \cdot 10^{-5}$
2-Chloro	0.23	0.69	0.41	-0.15	4.167	4.238	0.071	$5.78 \cdot 10^{-5}$
4-Chloro	0.23	0.93	0.41	-0.15	4.287	4.426	0.139	$3.75 \cdot 10^{-5}$
4-Bromo	0.23	1.13	0.44	-0.17	4.388	4.463	0.075	$3.44 \cdot 10^{-5}$
2,4-Dinitro[d]	2.02	0.06	1.34	0.32	4.572	4.591	0.019	$2.56 \cdot 10^{-5}$
4-Phenyl	0.01	1.91	0.08	-0.08	4.673	4.667	-0.006	$2.15 \cdot 10^{-5}$
2,4-Dichloro	0.46	1.62	0.82	-0.32	4.710	4.795	0.085	$1.60 \cdot 10^{-5}$
2,4,6-Tribromo	0.83	2.91	1.32	-0.51	5.461	5.403	-0.058	$3.95 \cdot 10^{-6}$

a σ values taken from 9c unless otherwise noted.

b π values taken from A. Leo, C. Hansch and D. Elkins, Chem. Rev., 71 (1971) 525.

c C is the molar LC_{50} concentration.

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Evaluation methods

Three or more tests (four dose levels for each test) were run for each compound under investigation.

The data treatment was completely computerized using least squares, however, for possible clarity it will be explained as if each test were plotted by hand.

For each compound a graph was prepared plotting log percent survivors vs. time in hours[14]. From this plot it was possible to determine the percent survivors, for each dose level, at 48 h. This tends to average the results and increase the repeatability factor involved with biological testing. It should also be pointed out that one can calculate anything from a 24 hr. to a 96 hr. LC_{50} using this one set of data. The 48-h percent survival figures were converted to probit values using probit transformation tables [15]. These values were plotted vs. log molar concentration. The log LC_{50} molar concentration can then be found by observing the log molar concentration value which corresponds to a probit value of 5. The probit values and log molar concentration values were introduced into a least squares computer program which calculated the LC_{50} and also gave the correlation of the line to the data points.

This method, for determining the 48-hLC_{50} value, has the advantage of a 4-day observation period. The inconsistencies which arise when the animals are counted only once (at 48 h) are therefore averaged out, resulting in greater reproducibility. As a measure of the reliability of the screening procedure, a structure-activity correlation was attempted for a series of phenols. The hope was, as in those correlation successfully carried out in pharmaceutical drug design[16-18], not to be able to predict absolute values, but to recognize trends within a given

structure,

The structure-activity correlation was approached using the precedures developed by HANSCH[16]. This method[19-23] attempts to correlate the BR with two parameters, the relative partition coefficient π, and the Hammett electronic substituent constant σ. The partition coefficients are evaluated using an n-octanol-water system, and π is defined as $\log P_X - \log P_H$ where P_H is the partition coefficient for phenol itself and P_X is the partition coefficient for a derivative.

The general form of the Hansch equation is shown below in Eqn. 1.

$$BR = a\pi^2 + b\pi\pi_0 + c\pi\pi_0^2 + d\sigma + e \quad (1)$$

One can expect to see simplified versions of qn. 1 depending on the relative importance of the parameters, π, σ, and the constant π_o. These modifications are given in Eqns. 2 through 6 (ref. 21).

$$BR = k_1\pi + k_2 \quad (2)$$

$$BR = k_1\pi^2 + k_2\pi + k_3 \quad (3)$$

$$BR = k_1\sigma + k_2 \quad (4)$$

$$BR = k_1\pi + k_2\sigma + k_3 \quad (5)$$

$$BR = k_1\pi^2 + k_2\pi + k_3\sigma + k_4 \quad (6)$$

It should be noted that Eqn. 4 considers the unlikely case where there is no dependency on π. A dependency on σ only would suggest a situation more easily visualized in an *in vitro* system rather than an *in vivo* one[20].

HANSCH et al., recently reported using a new set of parameters[24] F and R, which attempted to separate the inductive and resonance components of the substitutents. The additive nature of σ, for use in structure-activity correlations, has been demonstrated[9]. Therefore, due to the method by which F and R have been derived (from σ_m and σ_p), it appears reasonable to assume these parameters to be additive. Utilizing this

argument, the following two additional equations were included in the attempted correlation.

$$BR = k_1 \pi + k_2 F + k_3 R + k_4 \quad (7)$$

$$BR = k_1 \pi^2 + k_2 \pi + k_3 F + k_4 R + k_5 \quad (8)$$

Results

The data from the phenolic series in Table 1 were evaluated using the seven possible correlations (Eqns. 2 through 3). The results are listed below, where r is the correlation coefficient and c is the molar concentration at LC_{50}. The best correlations were obtained in Eqns. 7 and 8 which have both π, F and R dependency.

	r	
$\text{Log } 1/c = 0.527\ \pi + 3.796$	0.831	(9)
$\text{Log } 1/c = 0.059\ \pi^2 + 0.371\ \pi + 3.851$	0.835	(10)
$\text{Log } 1/c = 0.339\ \sigma + 4.129$	0.480	(11)
$\text{Log } 1/c = 0.173\ \sigma + 0.282\ \sigma + 3.914$	0.905	(12)
$\text{Log } 1/c = 0.062\ \pi^2 + 0.095\ \pi + 0.364\ \sigma + 3.613$	0.965	(13)
$\text{Log } 1/c = 0.500\ \pi + 0.453\ F + 0.637\ R + 3.731$	0.978	(14)
$\text{Log } 1/c = -0.028\ \pi^2 + 0.567\ \pi + 0.480\ F + 0.607\ R + 3.695$	0.978	(15)

The error analysis showed that Eqn. 14 represented the best correlation as the T values observed for the coefficients of the π^2 terms in Eqns. 10, 13, and 15 indicated these parameters added little or nothing to the correlation. The apparent improved correlation of Eqn. 13 over 12 is therefore not significant.

It must be emphasized that a correlation of this type is likely only if a series is picked in which the mode of death remains constant, and at best these correlations will only indicate trends. It can only be assumed that extreme deviations correspond to a change in the

mechanism of toxic action.

A significant observation should be noted in the context of the current evaluation of the relative merits of chlorination and ozonation as techniques for wastewater renovation and the previously observed incorporation of carbon-bound chlorine by a variety of phenolic systems under disinfection conditions; namely, increasing halogen substitution (i.e. enhanced lipophilic nature) in the phenol resulted in increased toxicity in agreement with the π, F and R dependency.

Part II

The availability of a rapid and accurate technique for the determination of partition coefficients (or their equivalent) became desirable when it was observed that the partition coefficient between n-octanol-water ($P_{o/w}$) was dominant in the successful Hansch correlation of phenol toxicity to aquatic species.[15] This concern has led to the development of a method using a permanently bonded long chain alkyl packing in a high pressure liquid chromatographic system and subsequently relating the capacity factor k´ $k' = (t_s - t_o)/t_o$, i.e. the net retention time relative to the nonadsorbed time to the partition coefficient $P_{o/w}$.

EXPERIMENTAL:

The separations were performed on 2-1/8" x 2' Bondapak C-18/Porasil B columns that were mounted in a Waters Associates ALC 202 (refractive index detector). The various mole percentages of distilled water and acetone (MCB-ACS grade) were eluted at 24-26°C and a flow rate of 0.9-1.0 milliliters per minute.

DISCUSSION:

It is well recognized that a separation (i.e. a difference in

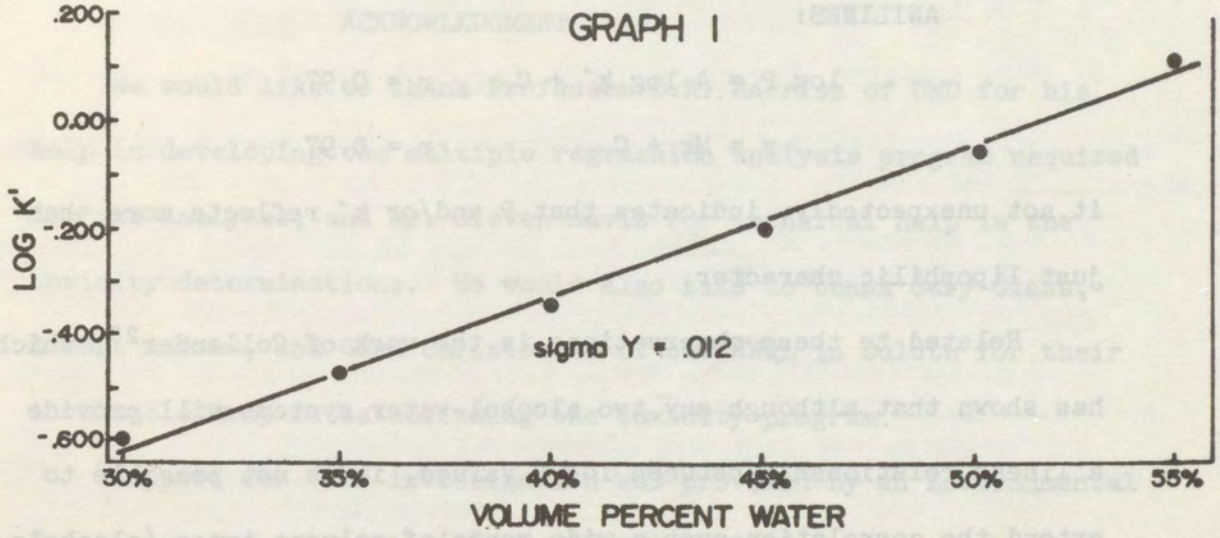
retention volume) in "reverse-phase" chromatography depends upon the partitioning characteristics of the solute between the mobile phase and the stationary phase as represented by the value of the partition coefficient. In the development of the overall approach for evaluating the elution data in the current study, it was therefore necessary to consider the relative number of moles of eluting solvent. However, the use of mole percents resulted in a significant increase in the deviation from linearity from that previously observed in the analysis of thin-layer partition data[26] when R_m [$R_m = \log (1/R_f - 1)$] was plotted vs. volume percent.

For example, Graphs 1 and 2 contain plots of log k′ vs. molar percent[27] (sigma y = 0.012) and log k′ vs. molar percent (sigma y′ = 0.035), respectively, for a representative compound (o-chlorophenol). It was subsequently found that the linearity could be maintained and substantially extended for all the compounds studied by plotting log (1/k′ + 1) vs. mole percent (e.g. o-chlorophenol, Graph 2, sigma y = 0.009). This change is valid as both the correlations of R_m and k′ are made over a range of percentages and the modification is therefore only from one empirical relationship to another.

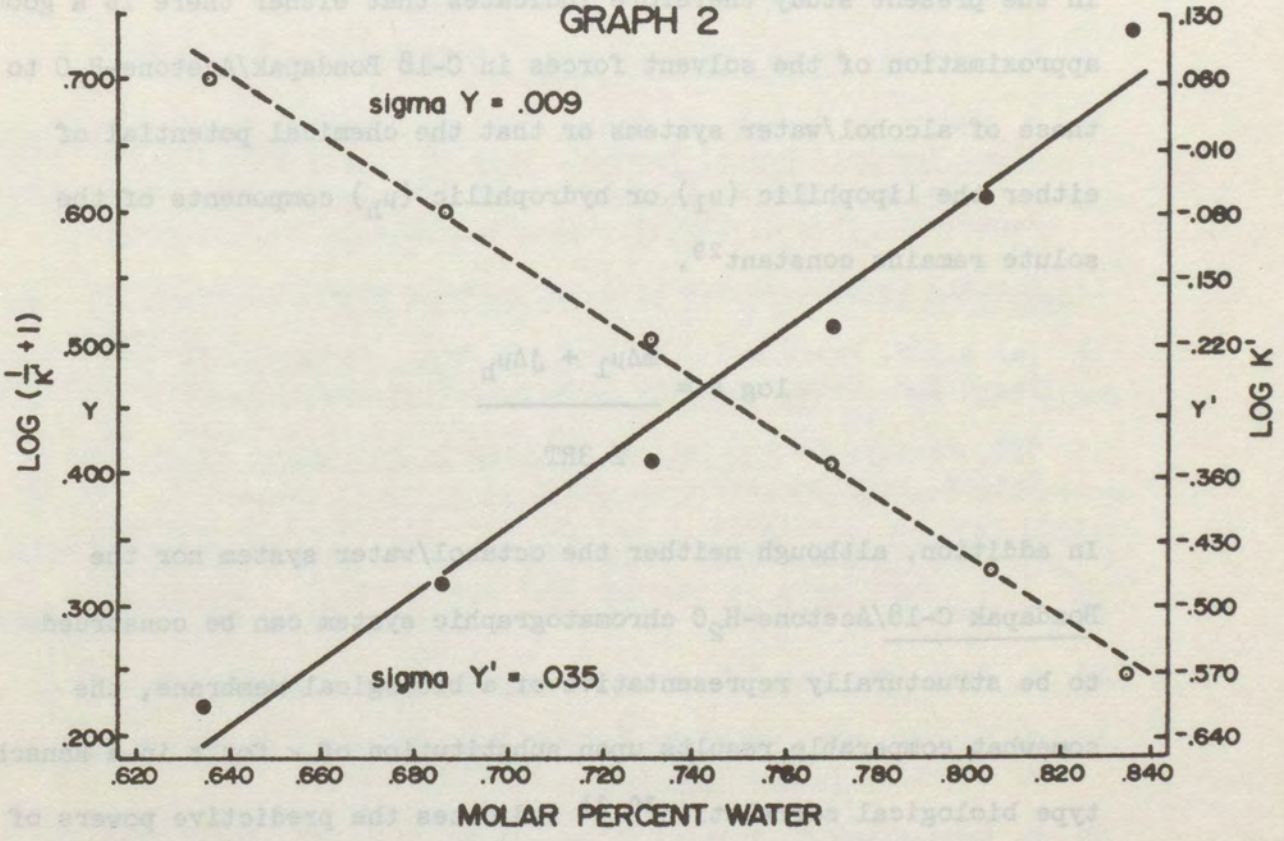
The correlations of log k′ to log P and π ($\pi = \log P_{\text{substituted}} - \log P_{\text{parent}}$) to κ ($\kappa = \log k'_{\text{substituted}} - \log k'_{\text{parent}}$) for some phenols and anilines are contained, along with their corresponding residuals, in Chart 2. The coefficients obtained from the individual regression analyses are found to be quite satisfactory. However, when the results from the two families of compounds are combined, the correlation of log P to log k′ decreases (r = 0.86). Although this is still an acceptable value for a regression analysis of this type,

RETENTION VOLUME OF O-CHLOROPHENOL

GRAPH 1



GRAPH 2



PHENOLS:

$$\log P = A \log k' + C \qquad r = 0.96$$

$$\pi = M\kappa + C \qquad r = 0.96$$

ANILINES:

$$\log P = A \log k' + C \qquad r = 0.97$$

$$\pi = M\kappa + C \qquad r = 0.97$$

it not unexpectedly, indicates that P and/or k′ reflects more than just lipophilic character.

Related to these observations is the work of Collander[28], which has shown that although any two alcohol-water systems will provide a linear relationship between log P values, it is not possible to extend the correlation over a wide range of solvent types (alcohols, esters, ketones, halogenated hydrocarbons). The successful correlation in the present study therefore indicates that either there is a good approximation of the solvent forces in C-18 Bondapak/Acetone-H_2O to those of alcohol/water systems or that the chemical potential of either the lipophilic (μ_l) or hydrophilic (μ_h) components of the solute remains constant[29].

$$\log P = \frac{m\Delta\mu_l + j\Delta\mu_h}{2.3RT}$$

In addition, although neither the octanol/water system nor the Bondapak C-18/Acetone-H_2O chromatographic system can be construed to be structurally representative of a biological membrane, the somewhat comparable results upon substitution of κ for π in a Hansch-type biological correlation[30,31] indicates the predictive powers of k′ and P in evaluating the ability of an organic molecule to pass

through biological tissue[32].

$$\log 1/c = M\pi + C \qquad r = 0.76$$

$$\log 1/c = M\kappa + C \qquad r = 0.68$$

ACKNOWLEDGMENT

We would like to thank Professor D.K. Harriss of UMD for his help in developing the multiple regression analysis program required for the analyses, and Mr. Steven Davis for technical help in the toxicity determinations. We would also like to thank Gary Glass, Robert Andrew, and Glen Christensen of the NWQL in Duluth for their invaluable help in establishing the toxicity program.

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27 $\log k' = R_m$

e.g. $k' = (t_R - t_o)/t_o = t_R'/t_o$

$R_f = t_o/(t_o + t_R') = \frac{1}{1+k'}$

$R_m = \log (1/R_f - 1) = \log k'$

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CHART 3 COMPARISON OF OBSERVED AND CALCULATED

BIOLOGICAL RESPONSES

Compound	Observed log 1/c*	Calculated log 1/c from κ	Calculated Log 1/c from π
Phenol	3.901	3.769	3.647
3-Methoxyphenol	3.480	3.710	3.715
4-Nitrophenol	4.219	3.845	3.952
4-Methylphenol	3.709	3.995	3.972
2-Methylphenol	3.835	4.073	3.979
2-Chlorophenol	4.238	4.065	4.114
2,6-Dimethylphenol	4.036	4.254	4.243
4-Chlorophenol	4.426	4.244	4.277
4-Bromophenol	4.463	4.356	4.412

*Toxicity to Dahpnia magna

THE IMPORTANCE OF BEING FAT SOLUBLE; AND UPTAKE, CLEARANCE, AND INTERTISSUE MOVEMENT OF HYDROPHILIC, AMPHIPHILIC AND HYDROPHOBIC COMPOUNDS

CHAPTER 4

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EDITOR'S REVIEW

A two compartment bio-energetics model for predicting bioaccumulation was discussed. Various rate constants are considered within this model and accumulation curves were shown by the use of theoretical calculations and laboratory data with DDT, PCB, methyl mercury and mercuric chloride.

This approach illustrated the necessity of considering the inter-relationship between various growth and energy factors within the bio-accumulation model:

1) uptake rate from water (Rpw)
   = (efficiency of extraction) X (concentration of pollutant) X (rate of passage of water through gills)
   The efficiency of extraction would be related to the n-octanol/water partition coefficient and the rate of passage of water through the gills, related to oxygen consumption.

2) uptake rate from food (Rpf)
   = (efficiency of extraction from food) X (concentration of pollutant in food) X (feeding rate)

3) rate of clearance of pollutant (R_{Cl})
   = (fractional clearance/kcal metabolic rate) X (body burden) X (total energy expenditure)

STRUCTURE-ACTIVITY RELATIONSHIPS OF DDT ANALOGS IN A NON-TARGET ORGANISM. LETHAL AND SUBLETHAL EFFECTS OF BROOK TROUT FINGERLINGS.

CHAPTER 5

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ABSTRACT

The raison d'etre, and the problems of the 'structure-activity' approach to toxicity are considered. Lethal and sublethal effects of 10 DDT analogs were estimated by mortality and temperature selection changes in brook trout fingerlings. The molecular requirements for lethality at 10-50 ppb differed from those that produced a change in temperature selection. The implications of these results for the 'structure-activity' approach will be discussed.

At a conference assembled to consider structure-activity correlations of toxicity and bioaccumulation in aquatic organisms, it seems possible to ask the question: 'What purpose does the approach of examining structure-activity relationships have?' I would suggest that answers to the question would include:

1) an attempt to elucidate the mechanism and/or site of action of a particular compound by comparison of in vitro and in vivo molecular requirements for toxicity, and by providing data on receptor site specificity, etc;

but also,

2) an attempt to eventually predict the toxicity of particular compounds by examination of their molecular structure and properties.

Although the experiments I will describe were primarily undertaken because of an interest in the first answer, I think the data will also tell us something about the reliability and wisdom of the second.

Of course, the idea that we should be able to predict the biological toxicity of a molecule by examination of its structure and properties is

simple, and therefore aesthetically attractive. Yet, as with so many things biological, the problems that confront us when we try to put this elegant idea into practice are immense. For instance: what biological parameters should we examine for structure-activity relationships; what can we examine with any degree of accuracy; what significance do these chosen parameters have to the survival of the whole animal. If we have to tackle the analysis with an in vitro approach, how do we relate that back to the in vivo situation. Obviously the answers to these questions are determined by the species of animal under study, together with the type of molecule being applied to it. That in itself raises yet another problem: how to confront the biological material with known concentrations of the compound under investigation. The problem can be one of accessibility to the site(s) of action in the animal (perhaps excluded by an impervious outer coast or by metabolic degradation), or, especially with hydrophobic compounds, getting known amounts reliably into an aqueous environment. This usually requires the aid of an organic solvent, which adds the concomitant problem of what effect it has on the biological material! (Of course, one attempts to clarify this by means of appropriate control experiments). Then there is the thorny subject of the mechanism(s) and/or site(s) of action of various compounds, about which, in many cases, we know precious little - and which, without accurate answers make any understanding and analysis of structure-activity relationships particularly difficult. Environmental factors can also affect the extent to which a molecule will produce a reaction, e.g., temperature, pH, water hardness, etc.: while from the animal's point of view there are, for instance, fat depots which can unleash stored toxic lipophilic molecules into the general circulation during times of starvation - an aspect that is

awkward to control experimentally. Then finally there is the problem of understanding the significance of the effects of various compounds at sublethal doses. How important to the animal's survival are the behavioural/physiological/biochemical parameters that can be altered by sublethal doses. Do they produce delayed mortality, reduced resilience, alter predator/prey relationships, upset breeding habits and success ? This question is particularly important in the case of that most unfortunate of animals, the non-target organism.

With these problems in mind I would like to describe and discuss some experiments performed to examine the effect of 10 DDT analogs on brook trout fingerlings; i.e., the relationship between a molecule of known pesticide properties and a non-target organism.

The first question that had to be asked was: what is the probable site of action of DDT in this organism? The effects of p,p'-DDT on fish - convulsions and frenetic movements - suggested that one site of DDT action was associated with the nervous system. This is corroborated for instance, by the experiments of Anderson (1968) who found that DDT caused lateral line hypersensitivity in trout; the elegant experiments of Narahashi and Haas (1968) which showed that DDT exposure altered lobster axon properties (with similar data recorded by Hille (1968) from frog Nodes of Ranvier); while Matsumura and O'Brien (1966) suggested a charge-transfer complex between DDT and some component of the nerve membrane. The nervous system therefore seems to be implicated, but the actual site of action of DDT is still unknown.

As electrophysiological examination of the trout fingerling nervous system wasn't technically feasible I decided to see if some behavioural expression of the nervous system couldn't be used as a 'bioassay' instead. It is now established that fish can sense temperature (see Gardner, 1973),

such that, if placed in a temperature gradient apparatus they will select a particular temperature most frequently, i.e., the selected temperature. This selected temperature has been found to be dependent on the acclimation temperature, i.e., the temperature at which the fish were maintained for 3 to 4 weeks prior to experimentation (Javaid (1967), Anderson (1971)). The mechanisms for temperature acclimation and selection are unknown but are presumably functions of the central nervous system. Certainly Greer and Gardner (1970) have evidence that trout brain contain temperature sensitive neurones - which agrees with the idea of central nervous system involvement. Javaid (1967) had shown that DDT treatment could alter the selected temperature of various salmonid fingerlings. Consequently this parameter was chosen as a 'bioassay' because of its simplicity and reasonable experimental repeatability.

But we also have to try and assess the importance of this parameter to the trout in its natural environment. It seems reasonable to assume that the fish's metabolism is geared to certain temperature limits for maximal efficiency; that the fish's speed of reaction to a predator/prey situation may depend on temperature; that its oxygen requirements may not be satisfied by too high a temperature; that its breeding habits may be upset with a consequent reduction in future population size. These seem reasonable assumptions, but the evidence for them appears to be slender, i.e., there is the possibility that 'temperature selection' may not be important for the fish's survival. Added to this are the problems of the apparatus itself: for instance does it only measure temperature selection?

The temperature gradient and methods of procedure are described and discussed in Gardner (1973). Brook trout fingerlings (Salvelinus fontinalis) were used.

Slide 1

The apparatus created a temperature gradient of 5-25°C in a horizontal body of flowing dechlorinated water. Single fish were placed in the trough and allowed to adjust. Their positions were recorded every 15 seconds to give a total of 120 readings. This was equated to temperature by using 5 temperature probes placed in the trough.

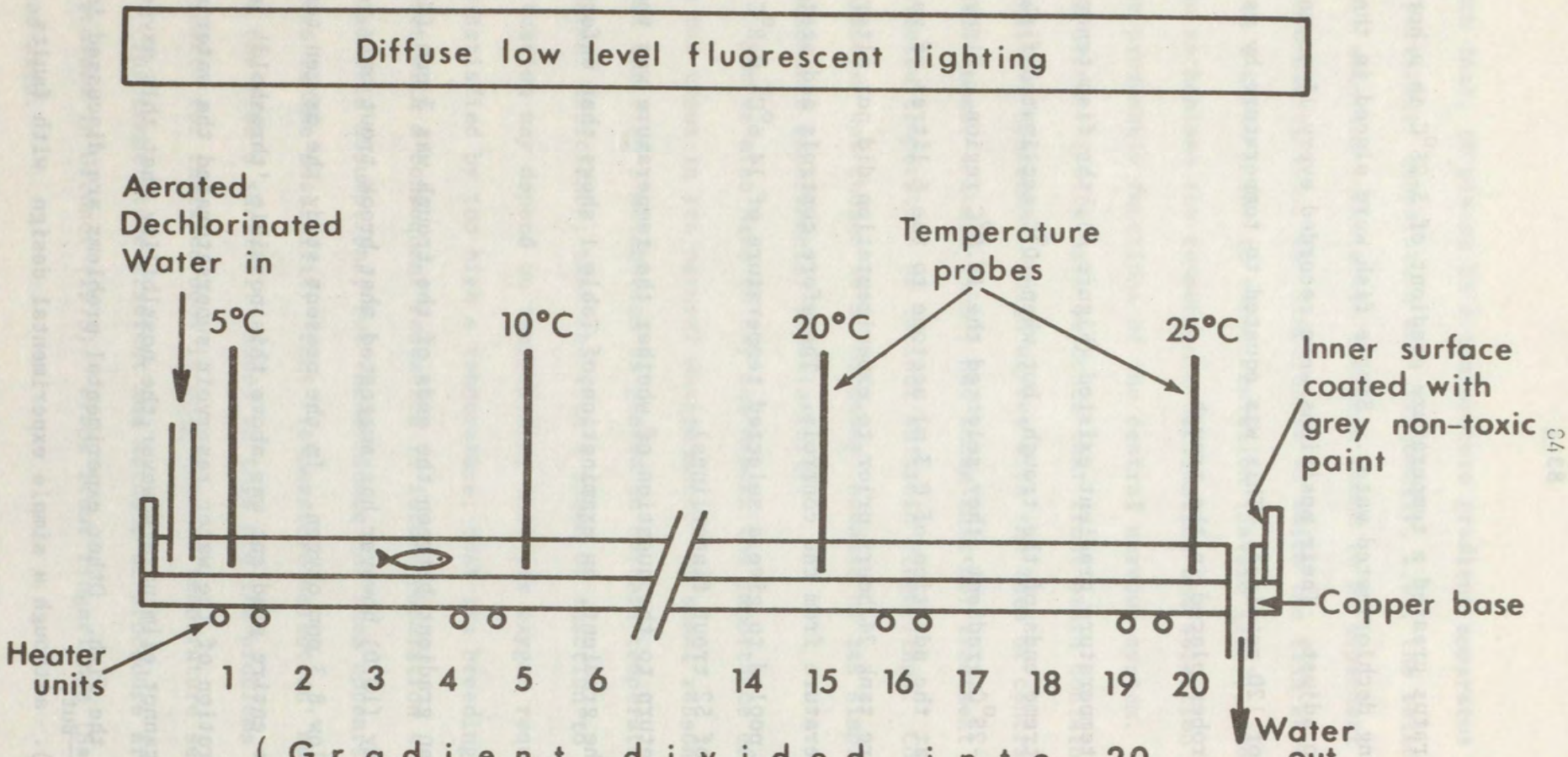
Slide 2

When no temperature gradient existed (Figure A), the fish tended to prefer the extreme ends of the trough, but when 10°C acclimated fish were placed in a 5-25°C gradient, they selected the 14.5°C region. Figures B and C show that the addition of 0.3 ml acetone to the 6 litres of water in the exposure tank 24 hours prior to experimentation did not alter the selected temperature from the controls. Therefore controls and acetone controls were pooled to give a selected temperature of 14.6°C $\pm$ 0.8°C (SD) (for a total of 52 trout fingerlings).

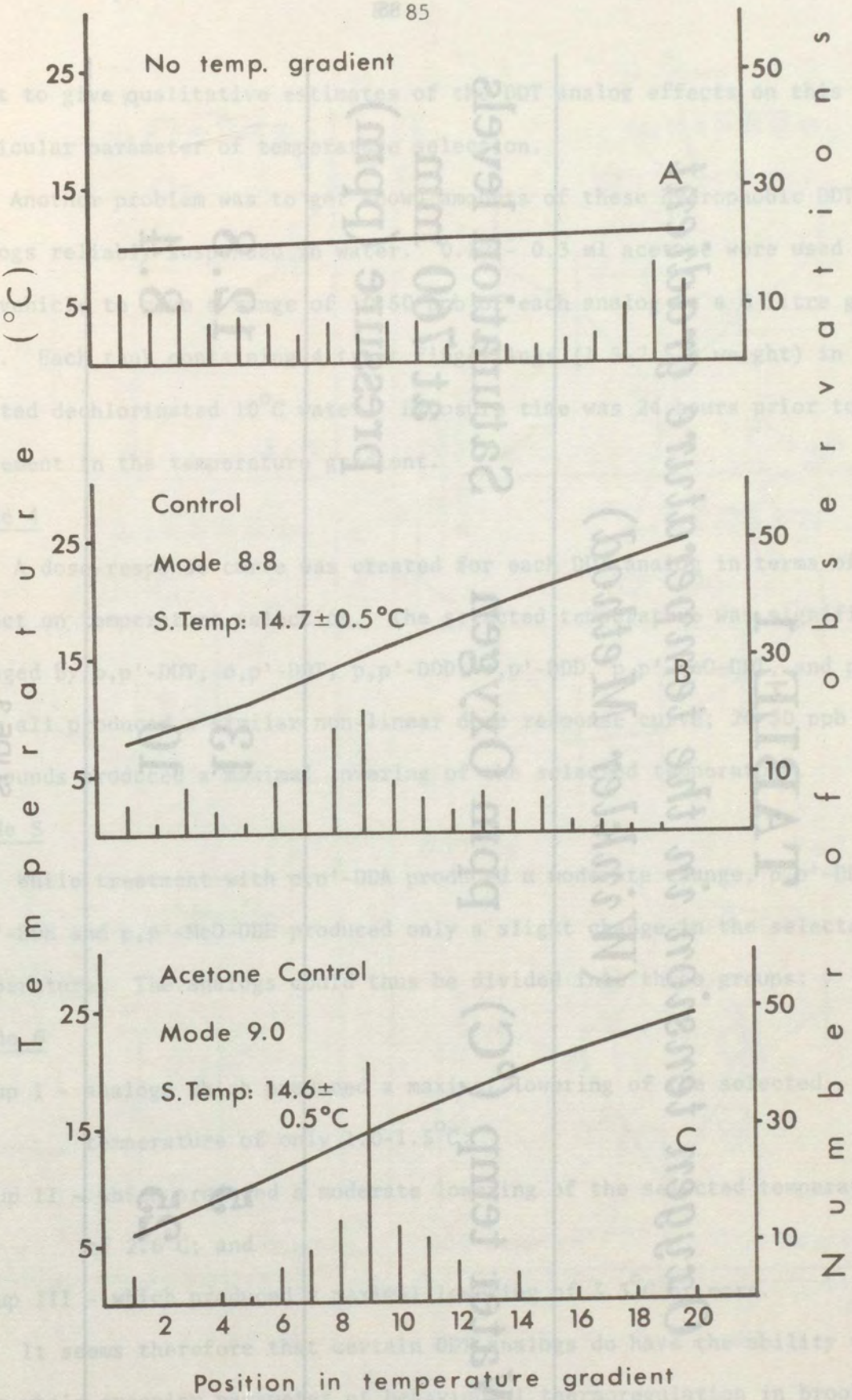
But to return to the question of whether the temperature was the only variable in the gradient, an examination of Table 1 shows that unfortunately it was not.

Slide 3

The oxygen gradient between the ends of the trough was 3 ppm (Winkler Method). Dandy (1970) however has suggested that brook trout only react to changes below 8.3 ppm oxygen. In the present study the oxygen tension throughout the entire gradient was above this possible 'threshold' level. Presumably aeration of the water reservoir supersaturated the water with oxygen. One cannot eliminate however, the possibility that this oxygen gradient did influence the fish. Other experimental problems are discussed in Gardner (1973). But, although a simple experimental design with faults, it did produce data that were relatively consistent. It seemed sufficient at


Diffuse low level fluorescent lighting
Aerated
Dechlorinated
Water in
5°C
10°C
20°C
25°C
Temperature
probes
Inner surface
coated with
grey non-toxic
paint
Copper base
Heater
units
1 2 3 4 5 6 14 15 16 17 18 19 20
Water
out
(Gradient divided into 20
reference compartments)

SLIDE 1


No temp. gradient
A
Control
Mode 8.8
S.Temp: 14.7±0.5°C
B
Acetone Control
Mode 9.0
S.Temp: 14.6±
0.5°C
C
25
15
5
50
30
10
(°C)
Temperature
Observations
Number
2 4 6 8 10 12 14 16 18 20
Position in temperature gradient

SLIDE 2

TABLE 1

Oxygen tension in the temperature gradient (Winkler Method)

Water temp (°C)	ppm Oxygen	Saturation levels at 760 mm pressure (ppm)
5	13	12.8
25	10	8.4

SLIDE 3

least to give qualitative estimates of the DDT analog effects on this particular parameter of temperature selection.

Another problem was to get known amounts of these hydrophobic DDT analogs reliably suspended in water. 0.12 - 0.3 ml acetone were used as the vehicle to give a range of 10-50 ppb of each analog in a 6 litre glass tank. Each tank containing 4 trout fingerlings (1.5-2.5 g weight) in aerated dechlorinated 10°C water. Exposure time was 24 hours prior to placement in the temperature gradient.

Slide 4

A dose-response curve was created for each DDT analog in terms of effect on temperature selection. The selected temperature was significantly changed by p,p'-DDT, o,p'-DDT, p,p'-DDD, o,p'-DDD, p,p'-MeO-DDT, and p,p'-Cl-DDT. They all produced a similar non-linear dose response curve; 20-30 ppb of these compounds produced a maximal lowering of the selected temperature.

Slide 5

While treatment with p,p'-DDA produced a moderate change, p,p'-DDE, o,p'-DDE and p,p'-MeO-DDE produced only a slight change in the selected temperature. The analogs could thus be divided into three groups:

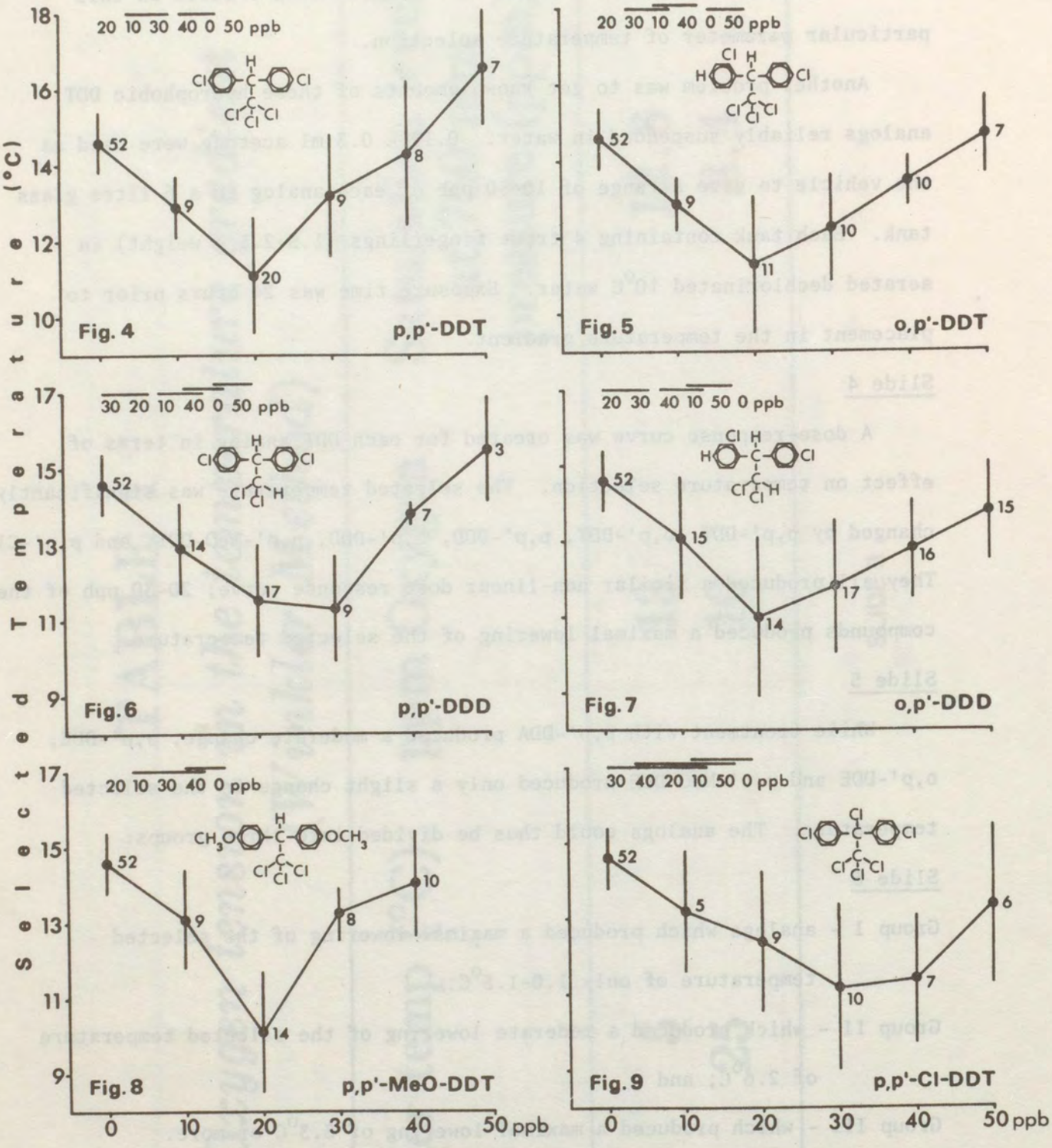
Slide 6

Group 1 - analogs which produced a maximal lowering of the selected temperature of only 1.0-1.5°C;

Group II - which produced a moderate lowering of the selected temperature of 2.6°C; and

Group III - which produced a maximal lowering of 3.3°C or more.

It seems therefore that certain DDT analogs do have the ability to alter this whole-organism parameter of behavioural thermoregulation in brook trout. All, except the DDE configurations, were effective at producing a change in selected temperature. A methoxy- rather than a chlorine-substitution of


Selected Temperature (°C)
20 10 30 40 0 50 ppb
52
9
20
9
8
7
Fig. 4
p,p'-DDT
20 30 10 40 0 50 ppb
52
9
11
10
10
7
Fig. 5
o,p'-DDT
30 20 10 40 0 50 ppb
52
14
17
9
7
3
Fig. 6
p,p'-DDD
20 30 40 10 50 0 ppb
52
15
14
17
16
15
Fig. 7
o,p'-DDD
20 10 30 40 0 ppb
52
9
14
8
10
Fig. 8
p,p'-MeO-DDT
30 40 20 10 50 0 ppb
52
5
9
10
7
6
Fig. 9
p,p'-Cl-DDT
0 10 20 30 40 50 ppb

SLIDE 4

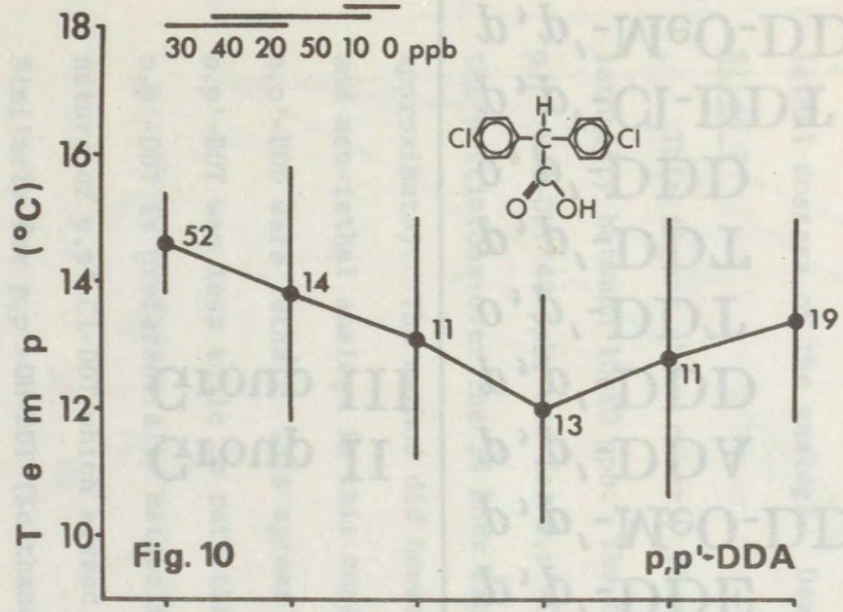


Fig. 10 p,p'-DDA

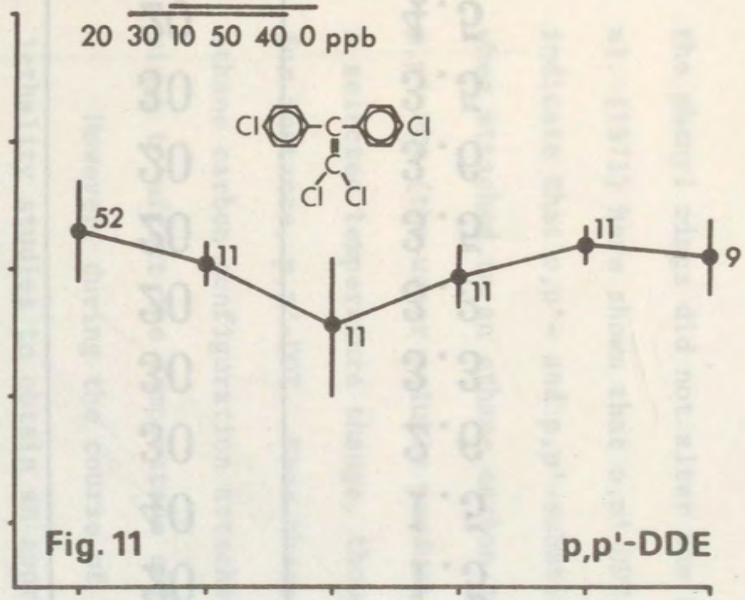


Fig. 11 p,p'-DDE

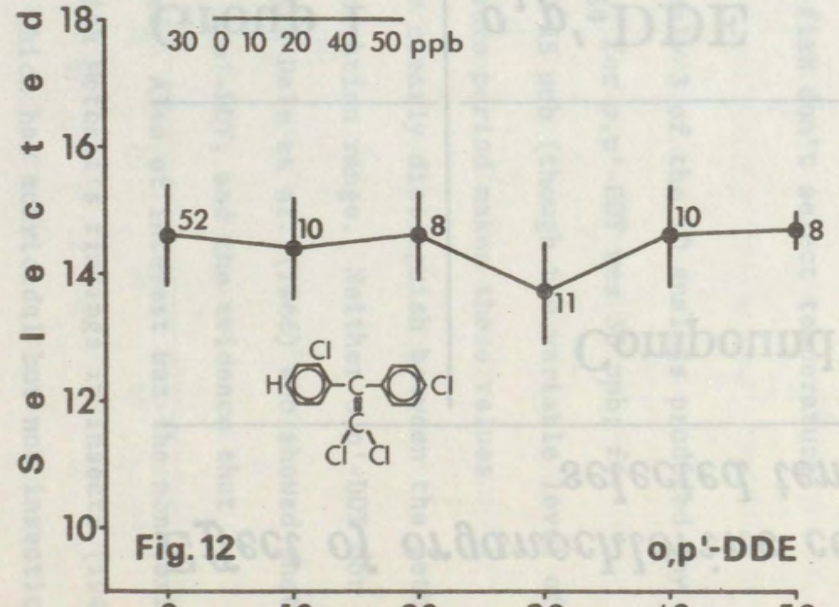


Fig. 12 o,p'-DDE

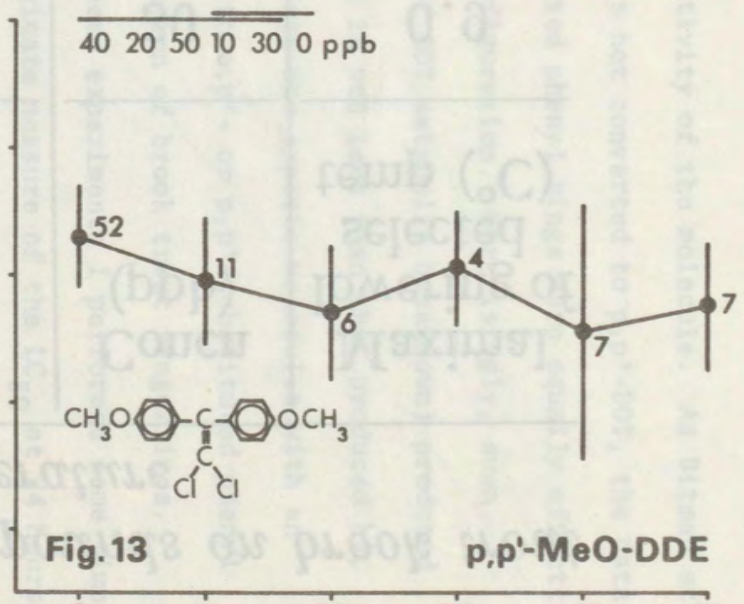


Fig. 13 p,p'-MeO-DDE

0 10 20 30 40 50 ppb

SLIDE 5

TABLE 2

Effect of organochlorine compounds on brook trout selected temperature

	Compound	Concn (ppb)	Maximal lowering of selected temp (°C)
Group I	*o,p'*-DDE	30	0.9
	p,p'-DDE	20	1.5
	p,p'-MeO-DDE	40	1.5
Group II	*p,p'*-DDA	30	2.6
Group III	*p,p'*-DDD	30	3.3
	o,p'-DDT	20	3.3
	p,p'-DDT	20	3.5
	o,p'-DDD	20	3.6
	p,p'-Cl-DDT	30	3.5
	p,p'-MeO-DDT	20	4.5

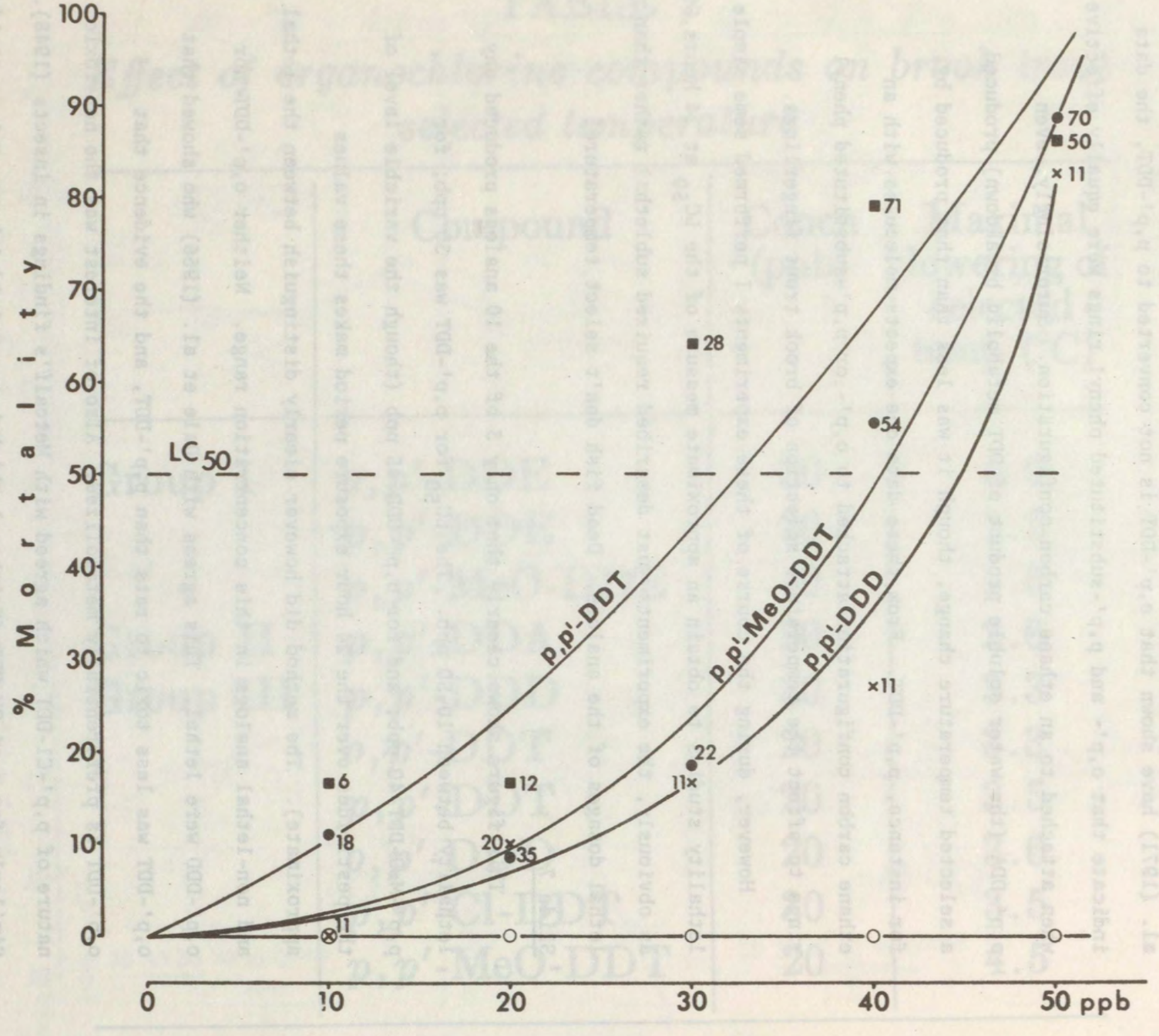
SLIDE 6

the phenyl rings did not alter the activity of the molecule. As Bitman et al. (1971) have shown that o,p'-DDT is not converted to p,p'-DDT, the data indicate that o,p'- and p,p'-substituted phenyl rings were equally effective when attached to an ethane carbon configuration. Surprisingly, even p,p'-DDA (the water soluble product of DDT metabolic breakdown) produced a selected temperature change, though it was less than that produced by, for instance, p,p'-DDT. From these data one expects molecules with an ethane carbon configuration attached to o,p'- or p,p'-substituted phenyl rings to affect the temperature selection of brook trout fingerlings.

However, during the course of these experiments I performed some simple lethality studies to obtain an approximate measure of the LC_{50} at 24 hours, as obviously, the experiments just described required sublethal rather than lethal dosages of the analogs. Dead fish don't select temperature!

Slide 7

This figure shows clearly that only 3 of the 10 analogs produced any lethality between 10-50 ppb. The LC_{50} for p,p'-DDT was 30 ppb; for p,p'-MeO-DDT 40 ppb; and for p,p'-DDD 45 ppb (though the variable level of the pesticides over the 24 hour exposure period makes these values approximate). The method did however clearly distinguish between the lethal and non-lethal analogs in this concentration range. Neither o,p'-DDT nor o,p'-DDD were lethal. This agrees with Dale et al. (1966) who showed that o,p'-DDT was less toxic to rats than p,p'-DDT, and the evidence that o,p'-DDT is preferentially metabolized. Also of interest was the non-toxic nature of p,p'-Cl-DDT which agreed with Metcalf's findings in insects (1948). Similarly for p,p'-OH-DDT (Kelthane) which has acaricidal but not insecticidal properties (Brooks, 1974). Perhaps the extra chlorine on the 2-carbon position shifts the charge distribution of the molecule, or it upsets the molecular geometry of the compound so that it no longer closely fits its site


100
90
80
70
60
50
40
30
20
10
0
% Mortality
LC50
P,p'-DDT
P,p'-MeO-DDT
P,p'-DDD
70
50
×11
71
28
54
×11
22
11×
6
12
18
20×
35
11
0
10
20
30
40
50 ppb

SLIDE 7

of action, as suggested by Mullins (1956), Holan (1969), and Fahmy (1973), or it strongly hinders free rotation of the molecular components (Riemschneider et al., 1954). Thus, the toxic action of p,p'-DDT seems to be a highly specific property of the molecule. The only alterations that do not seem to change the toxicity are exchange of methoxy for chlorine on the para positions of the phenyl rings, and the exchange of one of three chlorines on the 1-ethane carbon for a hydrogen (as in p,p'-DDD). The other alterations or additions to the molecule all produced non-toxic compounds at the concentrations tested.

Therefore we see that the molecular requirements, as deduced from the 10 analogs studied, to produce lethality and temperature selection are apparently entirely different. This indicates that they should be regarded as separate events, and not necessarily different expressions of the same effect. In other words, it warns against estimating the toxicity of a particular compound from only one parameter of a biological organism's response. In this particular case we have seen that apparently non-lethal compounds such as o,p'-DDT, o,p'-DDD, p,p'-DDA, p,p'-Cl-DDT did cause a change in the selected temperature parameter. This would, one presumes, have particular consequences for the fish's survival in its natural environment.

One ray of hope does emerge from this series of experiments however. The time course for the duration of the change in temperature selection after 20 ppb treatment with p,p'-DDT and p,p'-MeO-DDT was also examined.

Slide 8

Neither the acetone control nor the p,p'-DDT data changed significantly over the 9 days after treatment, but the p,p'-MeO-DDT effect apparently declined, until by Day 5, the trout were exhibiting a normal temperature selection. This suggests that either the p,p'-MeO-DDT molecule was broken

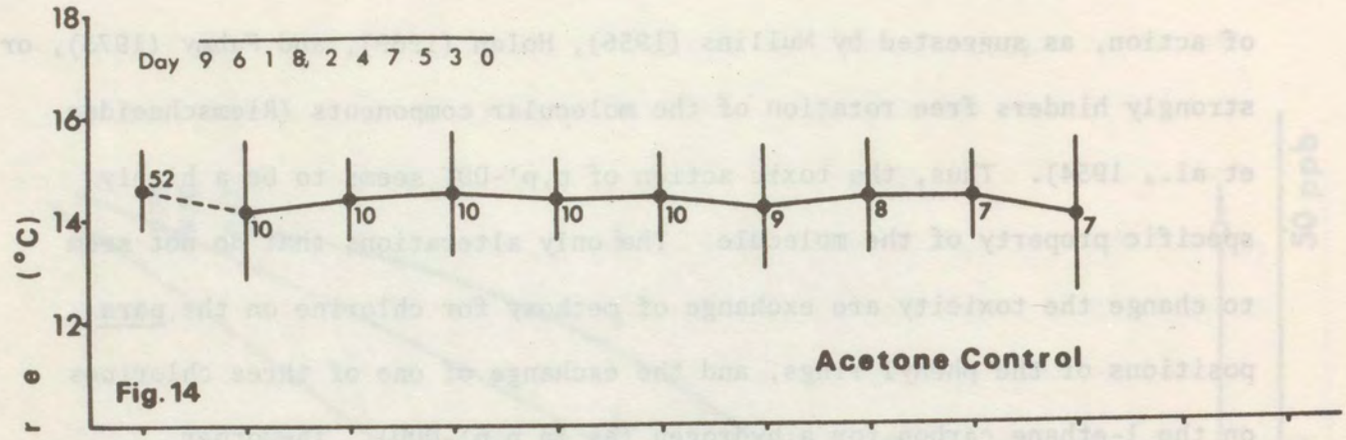


Fig. 14

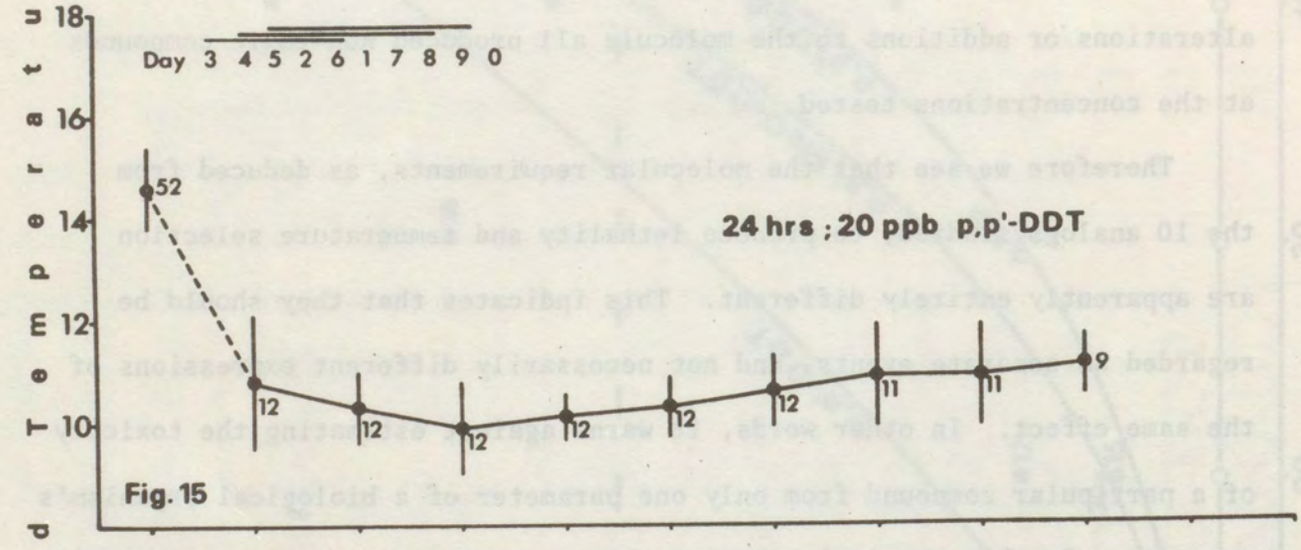


Fig. 15

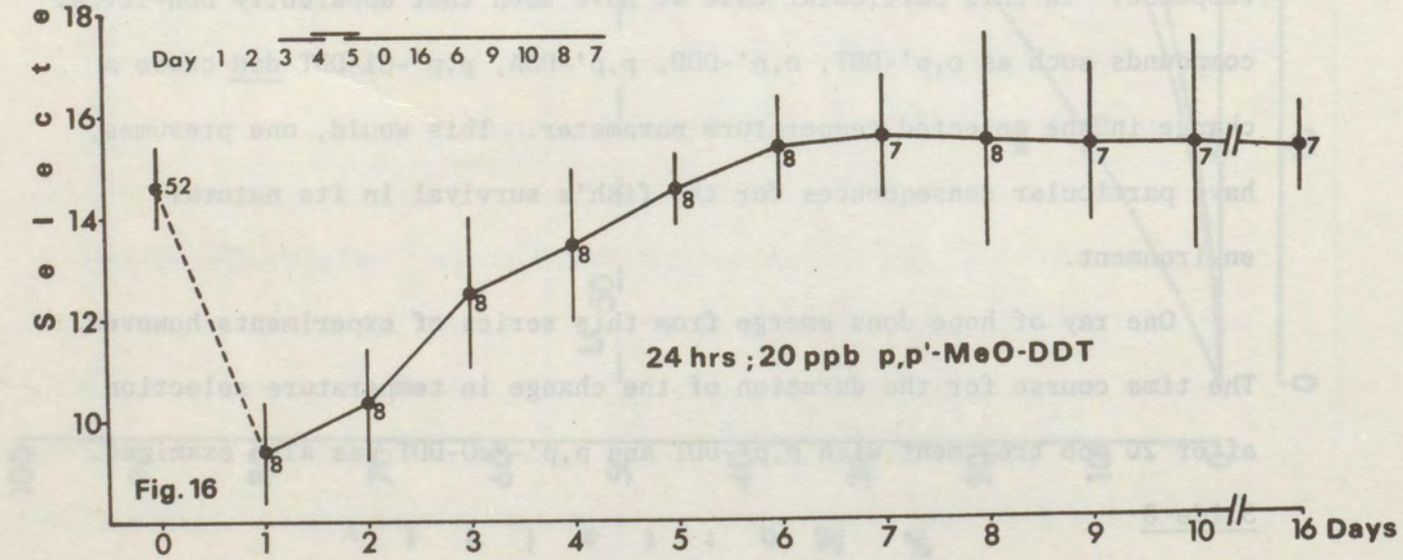


Fig. 16

SLIDE 8

down after absorption into the fish, or that the fish was able to adapt to its presence. The first possibility is in accord with the evidence that the p,p'-MeO-DDT molecule is biodegradable (Kapoor et al., 1970) and therefore less persistent than the p,p'-DDT. Biodegradability however is irrelevant if the trout suffers a lethal dose of p,p'-MeO-DDT!

In conclusion therefore, we must be careful to closely examine the biological parameters we choose as measures of structure-activity relationships for various compounds. Also, we need to remember that sublethal effects on target, and particularly non-target, organisms might eventually produce a delayed mortality - in which case we must pay more attention to the sublethal effects of supposedly non-toxic compounds.

It seems that we need to be more sure of the mechanism(s) and/or site(s) of action of pesticide molecules before we can make predictions of analog toxicity from molecular structure and properties.

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PREDICTING A BIOCONCENTRATION POTENTIAL OF ORGANIC CHEMICALS IN FISH FROM PARTITION COEFFICIENTS

CHAPTER 6

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ABSTRACT

The bioconcentration of several chemicals in trout muscle was found to follow a straight line relationship with partition coefficient. Bioconcentration in this paper is defined as the ratio of the concentration of the chemical between trout muscle and the exposure water measured at equilibrium. Partition coefficient has the usual meaning in that it is the ratio of the equilibrium concentration of the chemical between a nonpolar and polar solvent (in this case, n-octanol and water were the two solvents used). The relationship was established by measuring the bioconcentration in trout of a variety of chemicals over a wide range of partition coefficients. The following equation of the straight line best can be used to predice the bioconcentration of other chemicals from their partition coefficients:

Log (Bioconcentration factor) = 0.542 Log (Part. Coef.) + 0.124. The predicted values for bioconcentration potential apply to a specific test procedure and have been used primarily to compare various new chemicals.

The ability of some chemicals to move through the food chain resulting in higher and higher concentrations at each trophic level has been termed biomagnification or bioconcentration (Kenaga, 1972). The wide spread distribution of DDT (Burnett, 1971; Nature, 1972) and the polychlorinated biphenyls (PCB) (Gustafson, 1970) have become classic examples of such movement. From an environmental point of view this phenomena becomes important when the acute toxicity of the agent is low and the physiological effects to unnoticed until the chronic effects become evident. Due to the insidious nature of the bioconcentration effect, by the time chronic effects are noted, corrective action like terminating the addition of the chemical to the ecosystem, may not take hold soon enough to alleviate the situation before irreparable damage is done. It is for this reason that prior knowledge of the bioconcentration potential of new or existing chemicals is desired. The importance of bioconcentration is also recognized by the Environmental Protection Agency (EPA). For example, the ability of a material to build up in the environment has become one of the proposed criteria that this regulatory agency is using in establishing toxic pollutant effluent standards (Quarles, 1973).

In spite of the complexity of the reactions that are involved in the biomagnification process we felt it important to see if a simple relation could be established between the physiocochemical properties of a chemical and its ability to bioconcentrate. It was our belief that the partition coefficient would be the most logical parameter to examine in this connection. If a simple relation could be established it would be of great benefit in

planning the future direction of any development work on a new chemical and in directing research efforts to determine the ultimate fate and distribution of others.

MATERIALS AND METHODS

Chemicals - The following chemicals representing a wide range of partition coefficients, were evaluated: 1) 1,1,2,2-tetrachloroethylene, 2) hexachlorobenzene, 3) 2,2',4,4'-tetrachlorobiphenyl, 4) 2-biphenylyl phenyl ether, 5) diphenyl ether, 6) carbon tetrachloride and 7) p-dichlorobenzene. All materials were examined for purity by means of gas chromatography and found to be >99% pure.

Bioconcentration factor in fish - The method described by Branson et al (1974) was used to determine the bioconcentration factor in rainbow trout (Salmo gairdneri Richardson). This method is based on determining the ratio of the concentration of the chemical in trout muscle to the exposure water under steady state conditions. The trout were 12 cm in length and weighed 8-10 gms. and fed Purina #2 Trout Chow three times each day at a rate to insure vigorous feeding. A photo period of 16 hours daylight was maintained in the laboratory. Lake Huron water was dechlorinated by passage through activated carbon and cooled by refrigeration to 15°C. The analysis of the water before filtration was made according to standard criteria (Standard Methods, 1971) and is shown in Table I.

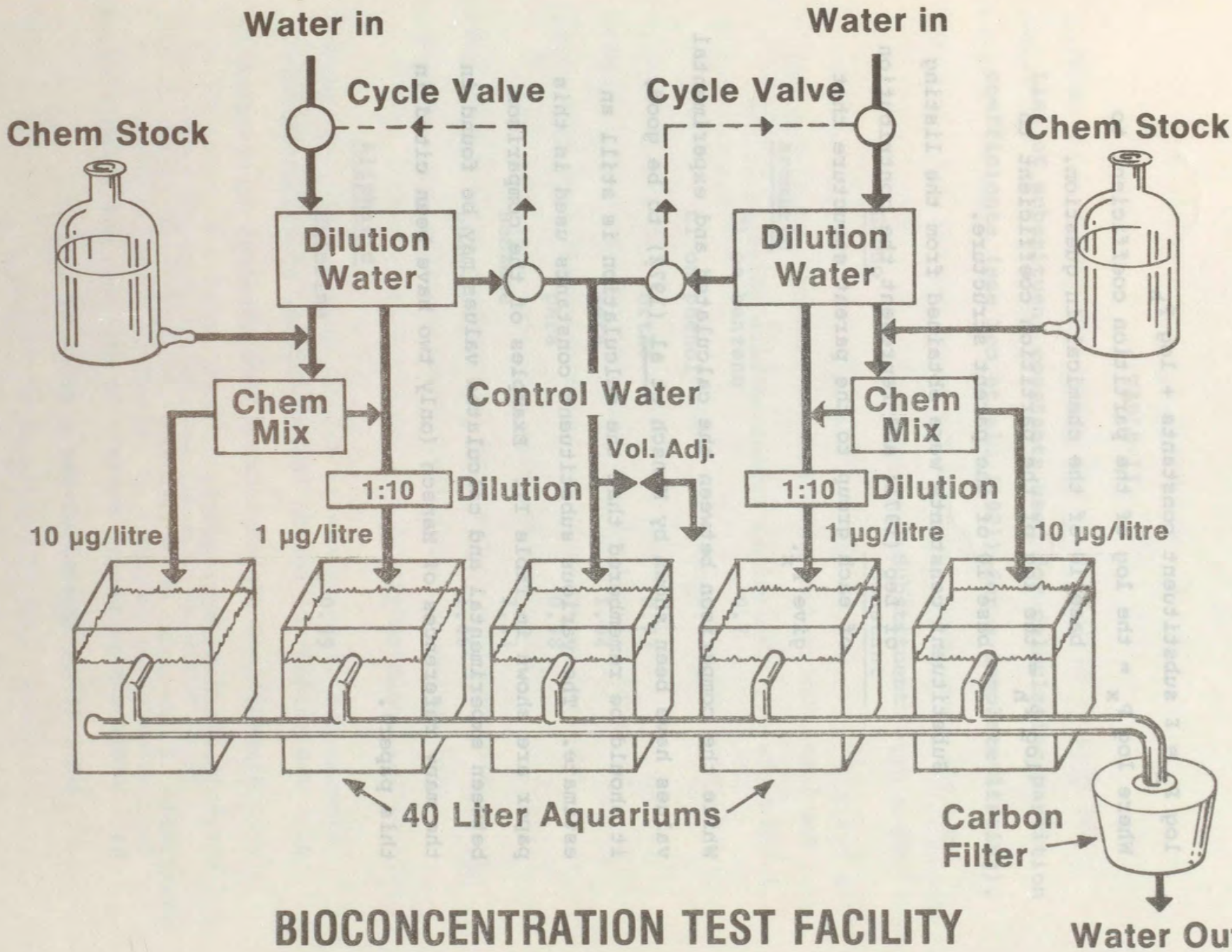
TABLE I

Chemical composition of Lake Huron water used in the bioconcentration studies.

Property	Value
pH	8
Total dissolved solids	150 mg/liter
Chloride	10 mg/liter
Calcium	27 mg/liter
Magnesium	7 mg/liter
Phosphate (as total P)	<0.1 mg/liter
Organic nitrogen	<0.4 mg/liter
Ammonia nitrogen	<0.05 mg/liter

Essentially there are three parts to the procedure; a) the concentration of chemical in the fish muscle is determined at various time periods during the uptake portion of the experiment. This is done by random sampling and sacrificing the fish from the bath; b) concentrations are determined in an analogous fashion during the clearance phase when the fish are placed in fresh water; c) the kinetic rate constants k_1 and k_2 describing the rate of uptake and clearance of chemical from the fish are estimated from the concentration time data via a nonlinear parameter estimation procedure (Draper and Smith, 1966) the ratio of these two estimates provides an estimate of the bioconcentration factor at steady state. The test equipment consisted of five aquaria, a constant temperature water bath and two proportional dilutors. Each dilutor as described by Mount and Brungs (1967) was constructed for delivery of two chemical concentrations each an order of magnitude apart. (Figure 1)

Partition coefficient - The partition coefficient of the chemical between n-octanol and water was either taken from the tabulation of Leo et al (1971) or calculated using the additivity principles as described by Hansch et al (1972) and illustrated below. The solvent system of n-octanol and water was used primarily because of the large accumulated data base that is available. A partition coefficient between n-octanol and water may be calculated using equation 1.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Chem Stock
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Dilution Water
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Chem Mix
Chem Mix
Control Water
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1:10 Dilution
10 µg/litre
1 µg/litre
1 µg/litre
10 µg/litre
40 Liter Aquariums
Carbon Filter
Water Out
BIOCONCENTRATION TEST FACILITY

FIGURE 1

$\log P_x = \Sigma$ substituent constants + $\log P_h$

Where $\log P_x$ = the log of the partition coefficient to base 10 of the chemical in question.

$\log P_h$ = the log of the partition coefficient to base 10 of the parent structure.

Substituent constants were obtained from the listing of Leo (1971) and represent the contribution of each group to the parent structure that give P_x.

While the comparison between the calculated and experimental values have been shown by Hansch et al (1972) to be good, it should be remembered that the calculation is still an estimate. The various substituent constants used in this paper are shown in Table II. Examples of the comparison between experimental and calculated values may be found in the many references of Hansch (only two have been cited in this paper).

TABLE II

List of substituent constants used for calculating partition coefficients (see Leo et al. (1971) for a complete listing).

Group	Substituent Constant
Aromatic	
Cl on benzene	0.7
Cl on phenol	
ortho	0.73
meta	1.04
para	0.98
Benzene	2.13
Phenol	1.46
Aliphatic	
Chlorine	0.39

RESULTS

The results of measuring the uptake and clearance rates of the various chemicals are shown in Table III. The values of the bioconcentration factor (log k_1/k_2) are shown in Table IV. The 95% confidence intervals for these factors was calculated by a Bayesian estimation procedure.

The logarithm of the partition coefficients are also given in Table IV. The values indicated were obtained in the following manner:

1. Tetrachloroethylene - An experimental value of 2.29 was obtained experimentally for trichloroethylene (Leo et al, 1971). These authors also indicated that a chlorine attached to a double bond is somewhere between an aliphatic and an aromatic chlorine. Consequently a value of 0.55 was added to 2.29.

2. The values for carbon tetrachloride, p-dichlorobenzene, diphenyl and diphenyl oxide were obtained experimentally (Leo and Hansch, 1971).

3. 2-Biphenylyl phenyl ether - To diphenyl was added a value of 1.46 for phenol giving a value of 5.55.

TABLE III

Results of measuring the uptake and clearance of various chemicals in trout muscle[1].

Chemical in exposure water	Uptake rate k_1 (hr^{-1})	Clearance rate k_2 (hr^{-1})	Bioconcentration k_1/k_2
1. 1,1,2,2-tetrachloroethylene	3.323±0.45	0.0823±0.030	39.6±5.5
2. Carbon tetrachloride	4.05±0.83	0.229±0.025	17.7±2.4
3. p-dichlorobenzene	5.670±0.425	0.0264±0.00157	215±21
4. diphenyl oxide	5.499±0.722	0.0280±0.0042	196±39
5. diphenyl	6.79±.52	0.0155±0.0012	438±48
6. 2-biphenylyl phenyl ether	8.06±0.715	0.0146±0.0025	552±107
7. hexachlorobenzene	18.76±0.78	0.00238±0.0004	7880±350
*8. 2,2',4,4'-tetrachloro diphenyl	11.9 ± 0.68	0.00125±0.0002	9530±1610

[1] These are the combined results of two separate experiments on each chemical at two different exposure levels.

* An abstract of a draft paper "Bioconcentration of 2,2',4,4'-Tetrachlorobiphenyl in Rainbow Trout as Measured by an Accelerated Test" by D. R. Branson, G. E. Blau, H. C. Alexander, D. R. Thielen and W. B. Neely, is included in Appendix 1 of this chapter.

TABLE IV

Bioconcentration factor in trout and the partition coefficients of the chemicals studied.

Chemical	log part. coeff.	log Bioconc. factor
1. 1,1,2,2-tetrachloro ethylene	2.88	1.59 (1.4-1.74)
2. Carbon tetrachloride	2.64	1.24 (1.16-1.30)
3. p-dichlorobenzene	3.38	2.33 (2.32-2.39)
4. diphenyl oxide	4.20	2.29 (2.23-2.34)
5. diphenyl	4.09	2.64 (2.59-2.68)
6. 2-biphenylyl phenyl ether	5.55	2.74 (2.64-2.81)
7. hexachlorobenzene	6.18	3.89 (3.80-4.07)
8. 2,2'4,4'-tetrachloro diphenyl	7.62	4.09 (4.00-4.16)

[1] Figures in parenthesis are nonsymmetrical 95% confidence limits.

4. Hexachlorobenzene - Four chlorine (2.8) were added to p-dichlorobenzene (3.38) to give 6.18 for this material.

5. 2,2',4,4'-Tetrachloro diphenyl - Values of 2x(0.73 and 0.98) was added to diphenyl to give a final result of 7.62.

The straight line of best fit was drawn through the points of partition coefficient and bioconcentration factor and is shown in Figure 2 with the equation for the line given in (2).

$$\log (\text{Bioconc. factor}) = 0.542 \log (\text{Part. coeff.}) + 0.124 \quad (2)$$

A multiple correlation coefficient of 0.948 and a standard error of 0.342 was obtained from the regression, an F test indicated a confidence level of 0.999. The 95% confidence region for the line is also shown in Figure 2.

DISCUSSION

As can be seen from Figure 2 and the resulting statistics a straight line can be used to represent the relationship between partition coefficient and bioconcentration factor. The 95% confidence limits on the values of bioconcentration factor predicted by the straight line are larger for values of the partition coefficient further removed from the mean value of

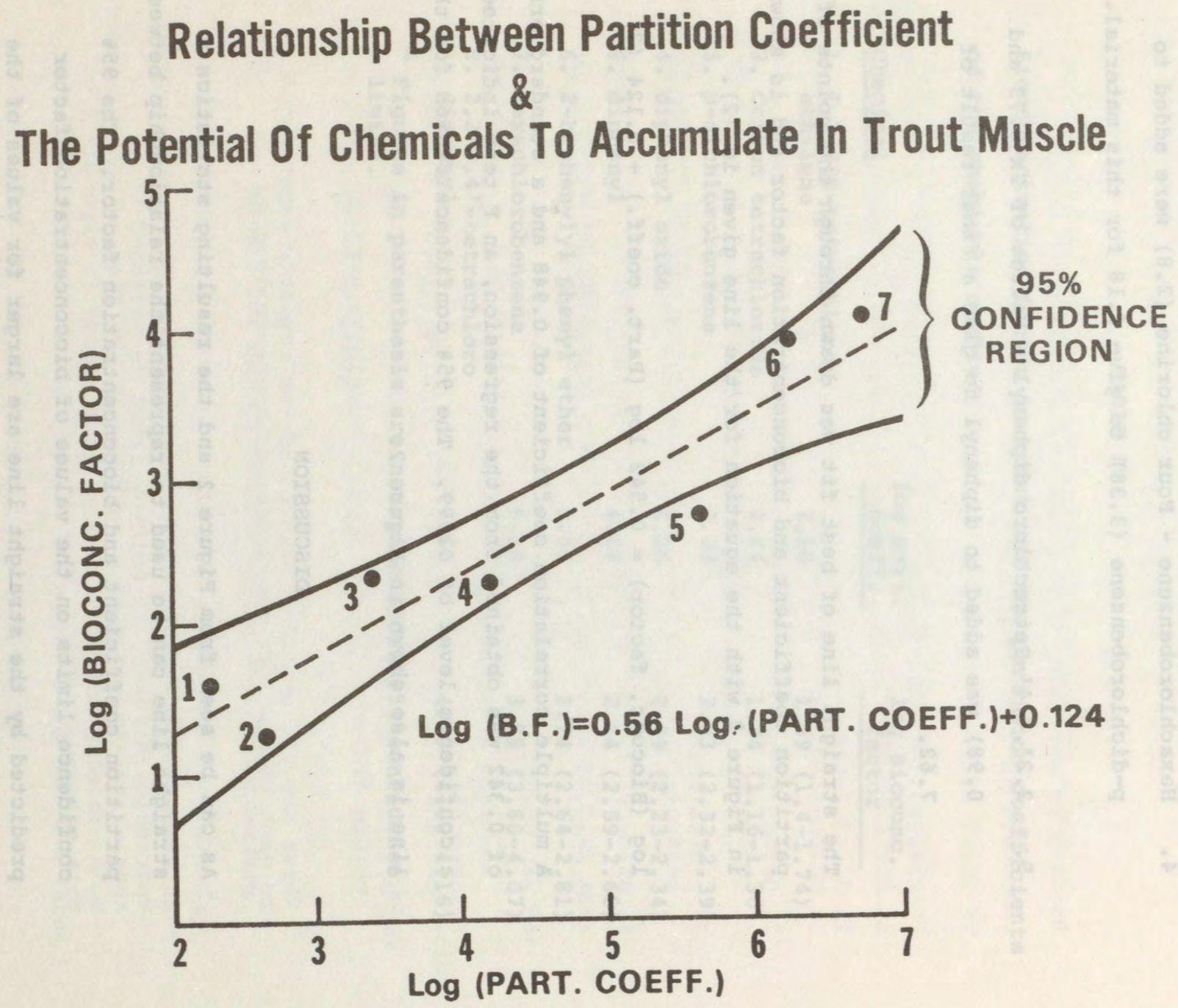
Relationship Between Partition Coefficient
&
The Potential Of Chemicals To Accumulate In Trout Muscle
Log (BIOCONC. FACTOR)
Log (PART. COEFF.)
95%
CONFIDENCE
REGION
Log (B.F.)=0.56 Log (PART. COEFF.)+0.124
1
2
3
4
5
6
7

FIGURE 2

the partition coefficient used to construct the straight line of best fit. Consequently, less confidence must be placed on any predictions from partition coefficients that fall outside the range of the data in Figure 2.

With this in mind, it is instructive to see how good equation 2 is for predicting the bioconcentration factor in fish. The values presented in Table V were taken from unpublished work at The Dow Chemical Company as well as from the literature (Ferguson et al, 1966). In both cases, the experimental procedure was slightly different and the fish species were mosquito fish (Gambusia affinia). It is rather striking, in view of these differences that such a close agreement between the experimental and calculated bioconcentration factor was observed. In interpreting the bioconcentration factor for chlorpyrifos or any chemical it must be remembered that metabolism for the agent may be a very active process (Smith et al, 1966). Consequently, the total amount of material in the ecosystem is constantly being reduced while the ratio between the fish and the environment will remain relatively fixed. The large standard deviation associated with the calculated value for pyridinol in Table V illustrates the dangers of departing too far from the mean of the regression line. Since the experimental bioconcentration factor for DDT was 5.23 (Hamelink, et al, 1971) and outside the region of the present regression, no attempt was made to make a prediction based on partition coefficient.

TABLE V

The use of regression equation 2 for predicting the bioconcentration factor.

Chemical	log (Part. coeff.)	log (Bioconc. factor)	
		Calculated	Experimental
Endrin	5.6[b]	3.47±.989[d]	3.17
Chlorpyrifos[a]	4.82[c]	2.87±0.963[d]	2.67
3,5,6-trichloro pyridinol	1.35[b]	0.88±1.139[d]	0.49

[a] 0,0-diethyl 0-(3,5,6-trichloro-2-pyridyl) phosphorothioate

[b] Calculated

[c] Experimental

[d] Standard deviation calculated from Draper & Smith (1966).

As more data is generated it will be important to modify the least squares parameter estimates of the straight line and recalculate the confidence regions. However, the present study does allow an investigator to begin rating the potential of new materials to concentrate. By matching this potential with the intended end use an early judgment decision can be made as to the possible long term environmental problems that may be faced.

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APPENDIX 1

Some hydrophobic chemicals may reach plateau levels in fish only after several months of continuous exposure. Therefore, an accelerated test procedure, based on kinetics, was developed using an isomer of PCBs (polychlorinated biphenyls); 2,2',4,4'-tetrachlorobiphenyl. The rates of uptake and clearance of 2,2',4,4'-tetrachlorobiphenyl were determined by analysis of rainbow trout (Salmo gairdneri Richardson). These trout were exposed to 1.6 and 9.0 µg/liter for five days and then transferred to fresh water. A nonlinear regression analysis was used to estimate the rate constants and to calculate the bioconcentration factor at steady-state. For 2,2',4,4'-tetrachlorobiphenyl, the extrapolated bioconcentration factor at steady-state was 9,530 ± 1,610 in trout flesh. This means, for example, that 5 µg/g in fish flesh with 2-4% fats and oils should be attained following exposure to 0.4 µg/liter (ppb) in water after 97 days.

The reliability of the bioconcentration factor based on this accelerated test procedure was checked with a 42-day test. The concentration of 2,2',4,4'-tetrachlorobiphenyl in trout flesh was 82 ± 20 µg/g after 42 days continuous exposure to 14 µg/liter. This was in good agreement with 92 ± 18 µg/g predicted from the accelerated procedure. The 42-day level was about 64% of that predicted at steady-state for 2,2',4,4'-tetrachlorobiphenyl in trout flesh. Therefore, this accelerated procedure yields more information in less time with similar accuracy about the potential of a chemical to bioconcentrate in fish.

BIOCONCENTRATION OF 14C-PESTICIDES BY BLUEGILL SUNFISH DURING CONTINUOUS AQUEOUS EXPOSURE

CHAPTER 7

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ABSTRACT

The rates of 14C-residue accumulation in the edible portion, the maximum (equilibrium) levels accumulated, and the rates of residue clearance, by bluegill sunfish (Lepomis macrochirus) have been determined for some fifty pesticides utilizing 14C-labelled materials. Fish were continuously exposed in dynamic systems, usually in two concentrations, for a minimum of 30 days, and then transferred for 14 days to uncontaminated flowing waters. Periodically during and after exposure, specimens were analyzed radiometrically to determine 14C-residue content.

Comparative data, providing indices of the relative propensity of different pesticides to accumulate and persist in bluegills are presented. These data are related to some existing information on other chemicals generally considered to pose a hazard to aquatic ecosystems. Consideration is given to relating water solubility of pesticides to propensity to bioaccumulate.

An alternative system for assessing similar parameters under more realistic environmental conditions is discussed and comparable data from both systems is compared.

INTRODUCTION

Recently, there has been great interest in the subject of the occurrence of chemical residues in fishes, the relative significance of direct aqueous exposure versus food chain contamination, and the persistence and toxicological significance of such chemical residues in fishes. The ability of fish to bioconcentrate chemical residues in their tissues above the concentration of the chemical in their aqueous environment has been clearly demonstrated (Macek, and Korn, 1970; Hansen et. al. 1971, Parrish et. al., 1975, Reinert et. al., 1974). Although the study of the phenomenon, of itself, does not provide one an assessment of the potential hazard to the environment associated with the use of such chemicals, information on uptake from water, and retention in tissues by fish, could prove a useful tool in assessing the relative propensity of a chemical to enter and persist in aquatic food chains.

This report describes the results of investigating the bioconcentration of more then fifty pesticides by bluegill, considers the trends indicated from these results, compares these data to similar information available for chemicals generally considered to present some hazard to the environment, and discusses the utility of such information as it relates to laboratory assessment of the ecological hazard associated with the introduction of chemicals into aquatic ecosystems.

Each of the chemicals was investigated under contract to the manufacturer of that chemical and the information generated during the study, and the specific conclusions generated therefrom are the property of the manufacturer. In view of the finite time frame in which this symposium was organized, it was impossible to obtain written permission from the manufacturer to release any or all of the data for specific chemicals. However, in view of the volume of information available at our laboratories, we considered presenting a general summary of our observations a worthwhile contribution to this program.

Also, an alternative system for assessing similar parameters under more realistic environmental conditions is discussed and comparable data for a pesticide assessed utilizing both systems is compared.

METHODS AND MATERIALS

Chemicals

In view of the inability to anticipate the occurrence, distribution and significance of all degradation products and/or metabolities found in even this simple system, we decided to utilize radiometric techniques to quantitate what might be considered "significant" chemical residues. We recognize that the decision as to which carbon atom (s) in the molecule is labeled is subjective to a certain extent; on the other hand we felt that more chemical residue could be quantitated more readily and more often by this technique than by monitoring the parent chemical and possibly known degradation products and/or metabolities by analytical methods specific for only these structures.

Exposure

Groups of bluegill sunfish (Lepomis macrochirus) having mean lengths ranging from 45-75 mm and mean weights of 2.5-5.0 g were obtained from commercial sources. All groups of fish were held in the hatchery facilities at Bionomics for a minimum of 30 days prior to use in any experiment. During that acclimation period, the cumulative mortality in any group was less than 3% and fish appeared to be in excellent physical condition prior to use.

Studies were conducted utilizing a modification of a continuous flow proportional dilution apparatus (Mount and Brungs, 1967) which provided for the automatic intermittent introduction of the 14C-pesticide and diluent water into each test chamber. Generally, three 60-liter glass aquaria containing 30 liters of test solution were utilized in each experiment. At the beginning of the experiment, one hundred (100) bluegill were placed into each aquaria. Aerated well water (pH 7.1, total hardness 35 mg/l as $CaCO_3$, dissolved oxygen >5.0 mg/l, temperature 20° C ($\pm$ 2) was provided to each aquaria at a flow rate of 5 l/hour. Fish were fed a dry pelleted ration ad libitum each day. Levels of exposure were selected on the basis of acute toxicity data and were intended to be sublethal during the continuous exposure period.

Sufficient 14C-labeled pesticide (~100 uc) was added to cold material to provide a specific activity sufficient to enable minimum detectable limits

in fish approximately 3 times the nominal concentration of the chemical in water. Specific activity for fish exposed to 1.0 mg/l of chemical generally ranged from 10-20 dpm/µg, while that for fish exposed to 0.01 mg/l of chemical ranged from 1000-2000 dpm/µg. The diluter was used prior to introduction of fish into experimental aquaria, to establish the desired chemical concentration, and after introduction of the fish to maintain that concentration.

Sampling Schedule and Techniques

Water and fish from each experimental unit, including controls, were sampled prior to the beginning of exposure and after 1, 3, 7, 10, 14, 21, 28 days of exposure (and every 7 days thereafter when necessary). Fish remaining in each aquaria at the end of the continuous exposure period were transferred to uncontaminated flowing water systems for 14 days to evaluate rates of 14C-residue elimination (depuration). During that period, fish were sampled 1, 3, 7, 10, and 14 days after transfer.

Duplicate five hundred (500) ml water samples were taken from each unit on all sample days during the exposure period. At each sampling interval (during both exposure and depuration) five (5) fish were removed from each experimental unit, eviscerated, and duplicate portions of the carcass (edible portion) analyzed radiometrically.

Radioassays

Duplicate samples of fish tissue (0.8-1.5 g) from each specimen samples were air dried for approximately 24 hours in combustion cones at 23°C. Each dried sample (0.5-1.0 g) was combusted in a Packard Model 306 Oxidizer, the resulting $^{14}CO_2$ was trapped as a carbonate in a mixture of a high capacity carbon dioxide absorber and toluene counting solution consisting of 8 g PPO + 0.25 g BIS-MSB/liter toluene. Standard reference material (14C-methyl methacrylate, 14,000 dpm/tablet) was analyzed with control fish tissue to determine recovery values from the oxidizer. Recovery values generally were quantitative ranging from 97-101%. Prior to the analysis of a series of tissue samples, the oxidizer was "cleaned" by consecutively combusting two pressed paper discs to eliminate any residual 14C-material (memory) which could be a source of error in analysis of low activity tissue samples.

Concentration of 14C-pesticide in water were usually determined by extracting duplicate 500 ml water samples with four-30 ml volumes of solvent (e.g. methylene chloride). The combined solvent extract was dried by passing it through sodium sulfate column followed by a solvent extraction of the column. The solvent was evaporated to 3-5 ml in a Kuderna-Danish evaporator then transferred to a scintillation vial and evaporated to dryness at room temperature. A xylene base counting solution (20 ml) consisting of nonionic surfactants with PPO + BIS/MSB scintillators was added to the vial and the sample was quantitatively divided into two equal subsamples each of which was analyzed radiometrically. For those few chemicals not readily extractable from water, concentration techniques involving slow evaporation of the water from the sample were utilized and recoveries determined. Recovery of 14C-pesticide from "spiked" water samples generally ranged from 70% to quantitative and, where necessary, results were corrected for recovery.

Counting Technique + Sensitivity

All measurements of radioactivity were made using a Model 2112 Packard Tri-Carb Liquid Scintillation Spectrometer to 4.5% probable error (95% confidence interval). Mean counting efficiency ranged from 68-82%. Efficiencies were determined by comparison with NBS calibrated 14C-toluene standards.

Mean background levels for untreated bluegill samples have been determined to be 47 CPM. Samples were counted for either 100 minutes or sufficient time to generate 5000 CPM. Utilizing this procedure, the probable error of accepting 15 CPM above mean background as minimum detectable limits was 0.05. Minimum detectable limits for water samples generally were equivalent to 1/10 the nominal concentration, and limits for fish samples (mg/kg) generally were 3X the concentration in water (mg/l).

RESULTS

Equilibrium

We have defined the equilibrium concentration as that mean tissue concentration (mg/kg) estimated from means, obtained at three successive sampling intervals, which do not statistically differ from each other. Alternatively, where the duration of the equilibrium is finite and sampling only identifies a real shift from the net rate of accumulation exceeding net rate of elimination to the reverse situation we have considered the maximum mean concentration (mg/kg) observed a valid estimate of the equilibrium concentration.

Bioconcentration Factors

We have calculated bioconcentration factors for each pesticide and the bluegill sunfish. The bioconcentration factor is defined as the ratio of mean 14C-residue concentration (mg/kg) in the muscle of bluegill at equilibrium to the mean concentration (mg/l) of 14C-pesticide in the aqueous environment during the total period of exposure prior to, and including the period in which steady-state is observed. Alternatively it can be defined as the ratio of the mean maximum 14C-residue (mg/kg) in muscle to the mean concentration (mg/l) of 14C-pesticide in the aqueous environment during the period in which the maximum tissue concentration is obtained. The two definitions yeild the same bioconcentration factor, although the latter may be more descriptive of situations relating to a maximum bioconcentration factor.

Biological Half-Life

The term half-life is a much utilized and often inaccurately applied term. In order to avoid misunderstanding, for the purposes of this discussion and the comparisons made herein, we have defined the"biological half-life" as the time required for bluegill containing an equilibrium concentration of 14C-residue in muscle to eliminate 50% of these residues upon transfer to an uncontaminated flowing water system.

Patterns of Accumulation

In general, we observed three basic patterns of accumulation of 14C-residue in muscle tissue by bluegill continuously exposed to a constant level of 14C-pesticide in flowing water. In the first instance, the net rate of accumulation exceeds the net rate of elimination throughout the period of exposure and thus tissue

concentrations continue to rise at what appears to be a linear rate (Figure 1). In the second instance, initially the net rate of accumulation exceeds net rate of elimination for a period of time, but eventually the rates become approximately equal and an equilibrium situation is established (Figure 2). It is assumed that until some externality occurs to shift the equilibrium, the tissue residue concentration will remain constant throughout continued indefinite exposure. In the last instance, the net rate of accumulation exceeds the net rate of elimination for a period, but eventually the rates become approximately equal. However, in this instance the equilibrium is very short lived, may in fact be virtually only for an instant, and then the net rate of elimination exceeds the net rate of accumulation, whereupon tissue residue declines despite continuous exposure (Figure 3). In these instances one can speculate that time-dependent or concentration-dependent enzyme induction processes could be significant.

Summary of General Observations

A representative number of general observations (exclusive of specific identification of the 14C-pesticides investigated) relating to the time to establish equilibrium, the bioconcentration factor obtained, the estimated biological half-life, and the effect of aqueous 14C-pesticide concentration on these parameters are summarized (Tables 1-3). For a great majority of the 14C-pesticides studied the data clearly indicate that an equilibrium is observed in a relatively short period of time (i.e. less then 3 weeks). We have observed this to occur with approximately 7 of every 10 pesticides investigated. For every 14C-pesticide we have investigated, we have observed equilibrium within the first 60 days of exposure.

As is evident from the data presented, we have observed a wide range (i.e. four orders of magnitude) of bioconcentration factors. However, none of the bioconcentration factors obtained are on the same order of magnitude as those reported for many chemicals (including pesticides) for which similar data describing accumulation of chemical residues in fish tissue are available. We have summarized the distribution of bioconcentration factors obtained for all of the 14C-chemicals we have investigated including several

FIGURE 1 - CONCENTRATION OF 14C-RESIDUES IN THE MUSCLE OF BLUEGILL SUNFISH CONTINUOUSLY EXPOSED TO 0.75 mg/1 OF A 14C-HERBICIDE IN WATER FOR 28 DAYS.

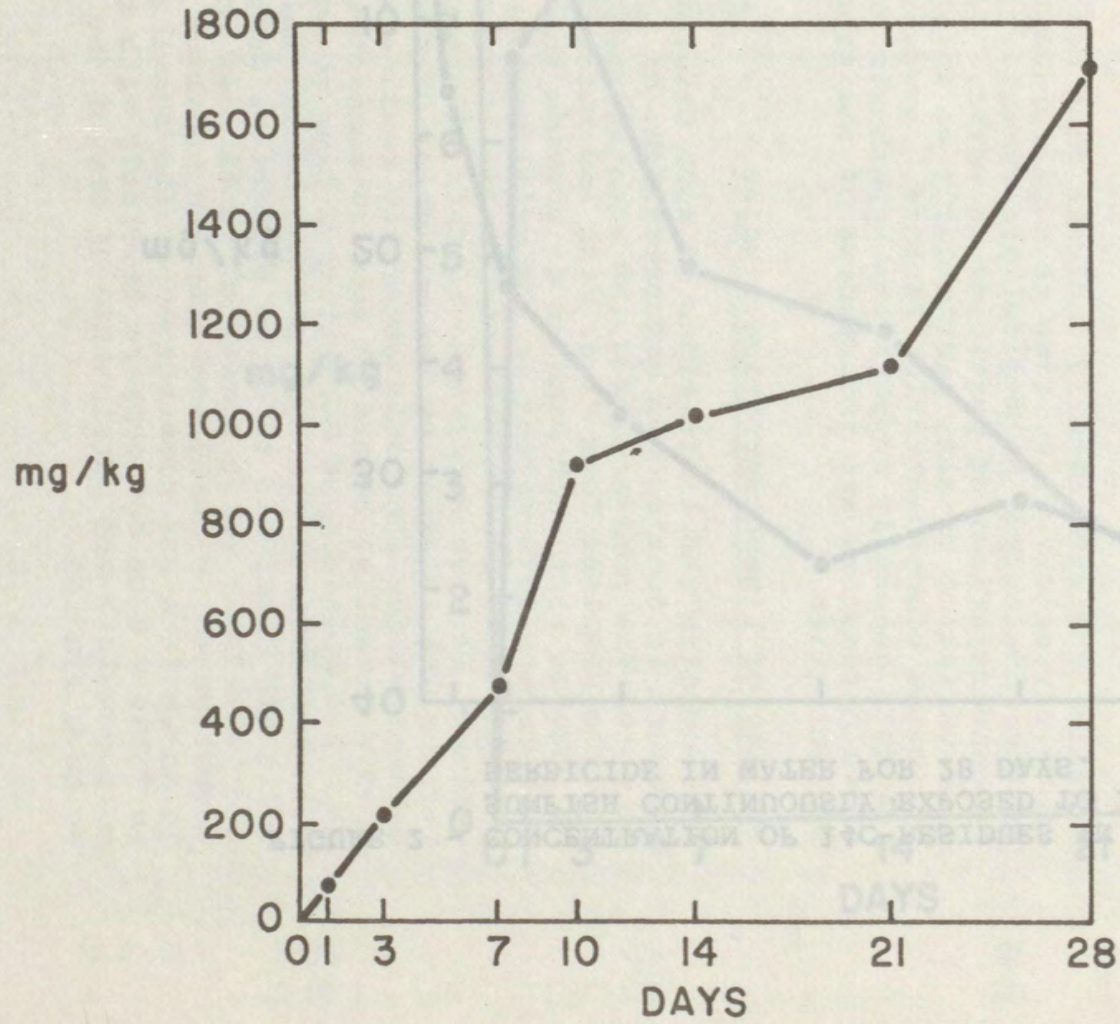


FIGURE 2 - CONCENTRATION OF 14C-RESIDUES IN THE MUSCLE OF BLUEGILL SUNFISH CONTINUOUSLY EXPOSED TO 1.0 mg/l OF A 14C-HERBICIDE IN WATER FOR 28 DAYS.

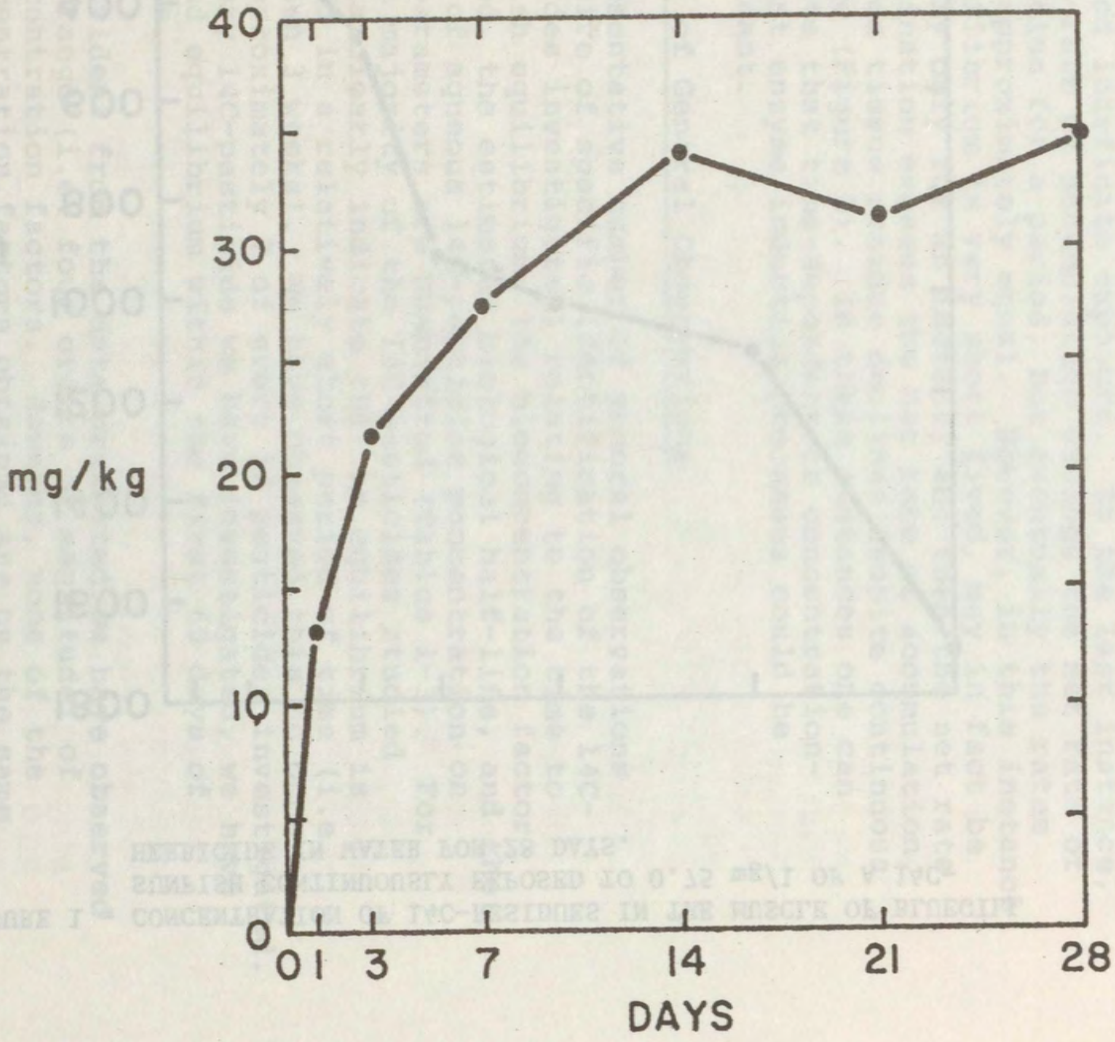
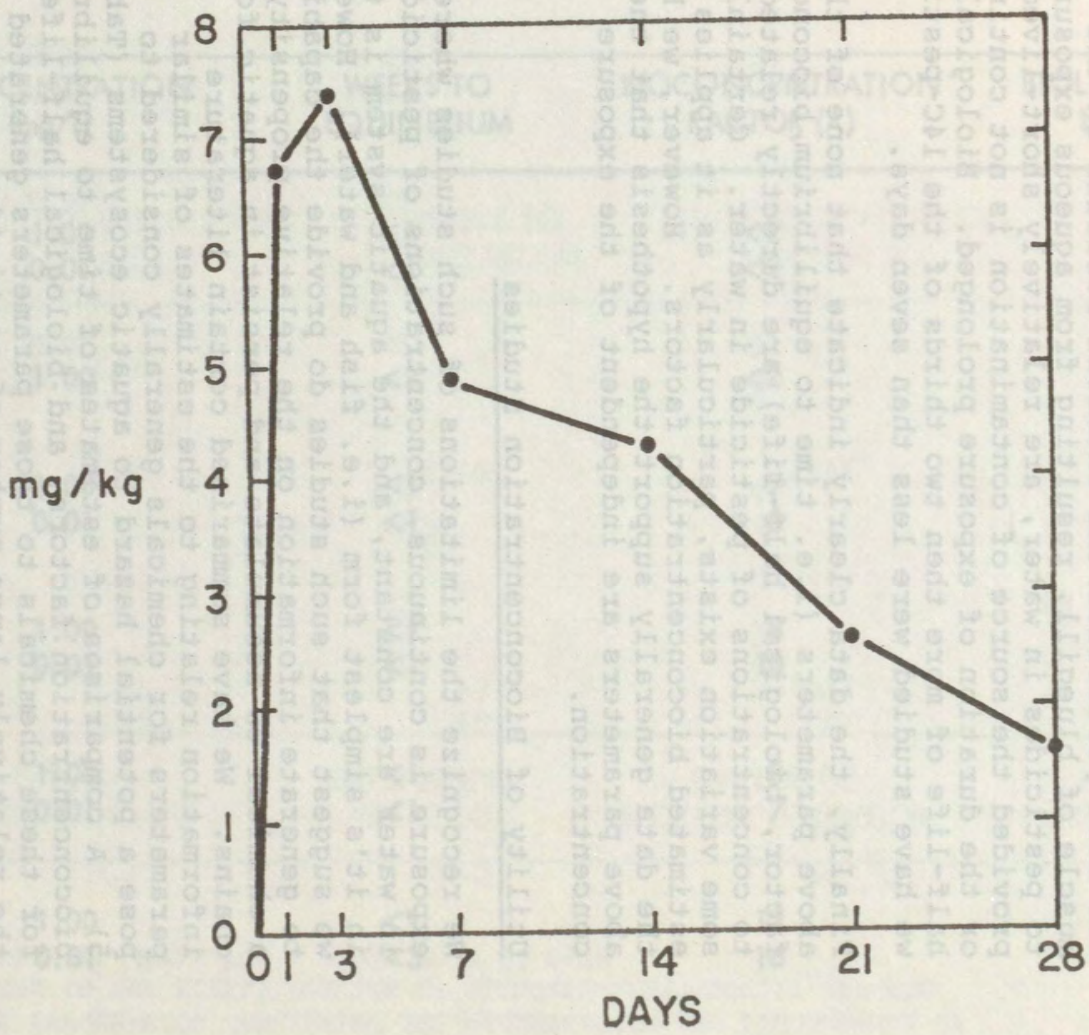


FIGURE 3 - CONCENTRATION OF 14C-RESIDUES IN THE MUSCLE OF BLUEGILL SUNFISH CONTINUOUSLY EXPOSED TO 1.0 mg/l OF A 14C-HERBICIDE IN WATER FOR 28 DAYS.



not utilized as pesticides (Table 4). Clearly, the data suggest that bioconcentration factors obtained for the majority of chemicals will range from 10-1000X with bioconcentration factors for only a relatively small minority of organic chemicals being <5X or >1000X.

The data presented (Tables 1-3) clearly suggest that for most pesticides, the chemical residues in the muscle of bluegill, resulting from aqueous exposure to pesticides in water, are relatively short-lived provided the source of contamination is not continuous or the duration of exposure prolonged. Biological half-life of more then two thirds of the 14C-pesticides we have studied were less than seven days.

Finally, the data clearly indicate that none of the above parameters (i.e. time to equilibrium, bioconcentration factor, biological half-life) are directly related to concentrations of pesticide in water. Certainly some variation exists, particularly as it applies to estimated bioconcentration factors. However, we believe the data generally support the hypothesis that the above parameters are independent of the exposure concentration.

Utility of Bioconcentration Studies

We recognize the limitations of such studies where exposure is continuous, concentrations of pesticides in water are constant, and the aquatic system is taken in it's simplest form (i.e. fish and water). However, we suggest that such studies do provide the capability to generate information on the relative propensity of a chemical to accumulate and persist in aquatic food chains. We have summarized certain literature information relating to the estimates of similar parameters for chemicals generally considered to pose a potential hazard to aquatic ecosystems (Table 5). A comparison of estimates of time to equilibrium, bioconcentration factors, and biological half-life for these chemicals to those parameters generated for the relatively large number of pesticides we have investigated, clearly suggests the relative propensity to accumulate and persistence of the chemicals presented in Table 4 does not compare favorably with the propensity to accumulate and persistence of any of the materials we have studied.

TABLE 1 - SUMMARY OF INFORMATION CONCERNING THE ACCUMULATION AND PERSISTENCE OF 14C-RESIDUES IN THE EDIBLE PORTION OF BLUEGILL CONTINUOUSLY EXPOSED TO 14C- PESTICIDE IN WATER FOR A MINIMUM OF 28 DAYS.

PESTICIDE	CONCENTRATION (mg/l)	WEEKS TO EQUILIBRIUM	BIOCONCENTRATION FACTOR (X)	BIOLOGICAL HALF LIFE (DAYS)
	1.00	<1	<2	>14
	0.01	<1	<2	>14
	1.00	<2	<2	<3
	1.00	<1	7	<3
	0.01	<1	5	<3
	1.00	<1	8	<1
	0.01	<1	10	<3
	1.00	<1	8	<4
	0.01	<1	7	<4
	1.00	<1	12	<1
	0.01	<3	16	<1
	1.00	<1	32	<7
	0.01	<1	33	<3

TABLE 2 - SUMMARY OF INFORMATION CONCERNING THE ACCUMULATION AND PERSISTENCE OF 14C-RESIDUES IN THE EDIBLE PORTION OF BLUEGILL CONTINUOUSLY EXPOSED 14C-PESTICIDE IN WATER FOR A MINIMUM OF 28 DAYS.

PESTICIDE	CONCENTRATION (mg/l)	WEEKS TO EQUILIBRIUM	BIOCONCENTRATION FACTOR (X)	BIOLOGICAL HALF LIFE (DAYS)
	0.50	<4	34	>28
	0.01	<4	22	>28
	1.00	<1	61	<1
	0.01	<1	45	<1
	0.50	<2	34	<1
	0.05	<2	73	<2
	0.25	<4	51	>28
	0.01	<4	113	>28
	0.20	<2	6	>14
	0.01	<2	37	>14
	1.00	<2	50	<3
	0.01	<2	9	<3
	0.10	<1	5	<7
	0.02	<2	22	<14

TABLE 3 - SUMMARY OF INFORMATION CONCERNING THE ACCUMULATION AND PERSISTENCE OF 14C-RESIDUES IN THE EDIBLE PORTION OF BLUEGILL CONTINUOUSLY EXPOSED TO 14C-PESTICIDE IN WATER FOR A MINIMUM OF 28 DAYS.

PESTICIDE	CONCENTRATION (mg/l)	WEEKS TO EQUILIBRIUM	BIOCONCENTRATION FACTOR (X)	BIOLOGICAL HALF LIFE (DAYS)
	0.025	<3	161	<14
	0.005	<3	145	<1
	0.005	<1	190	<3
	0.50	>3	>267	<3
	0.01	<2	267	<3
	0.25	<5	778	<3
	0.01	<5	279	<1
	0.05	<4	1040	<7
	0.01	<1	620	<7

TABLE 4 - DISTRIBUTION OF OBSERVED BIOCONCENTRATION FACTORS FOR MORE THEN FIFTY PESTICIDES AND BLUEGILL CONTINUOUSLY EXPOSED TO 14C-LABELED CHEMICAL IN WATER FOR A MINIMUM OF 28 DAYS.

	BIOCONCENTRATION FACTORS				
RANGE (X)	<5	>5 <10	>10 <100	>100 <1000	>1000*
OBSERVED (%)	7.5	17.5	42.5	25.0	7.5

* HIGHEST BIOCONCENTRATION FACTOR OBSERVED < 2000

TABLE 5 - SUMMARY OF INFORMATION RELATING TO BIOCONCENTRATION BY, AND BIOLOGICAL PERSISTENCE IN, FISHES OF SOME "PROBLEM" CHEMICALS IN AQUATIC ENVIRONMENTS.

CHEMICAL	CONCENTRATION (μg/l)	SPECIES	DAYS TO EQUILIBRIUM	BIOCONCENTRATION FACTOR (X)	HALF-LIFE (DAYS)	DATA SOURCE
DDT	0.003	TROUT	>120	8,500	ca 160	MACEK & KORN, 1970 - MACEK et al, 1970
DIELDRIN	2.0	TROUT	140	10,000	ca 40	BIONOMICS, 1974 MACEK et al, 1970
HEPTACHLOR	2.0	MINNOW	-	>10,000	<28	MACEK et al, 1975 ANDREWS et al, 1966
HEXACHLORO-BENZENE	5.0	PINFISH	14	>20,000	>60	PARRISH, 1974
AROCLOR 1254	1.0	SPOT	28	37,000	ca 42	HANSEN et al, 1971
DIOXIN (TCDD)	0.24	CATFISH	-	12,000	-	EISENSEE & JONES, 1975
METHYL MERCURY	0.25	TROUT	> 84	8,000	-	REINERT et al, 1974
TETRACHLOROBI-PHENYL	14.0	TROUT	> 48	12,400	30	BRANSEN et al, 1974
TOXAPHENE	0.5	TROUT	> 140	16,000	> 50	MAYER et al, 1974

The occurrence of such unusual deviations from the general ranges observed for these parameters could provide a useful screening mechanism for identifying that evidently small percentage of chemicals which may require careful and detailed assessment of potential hazards to aquatic ecosystems.

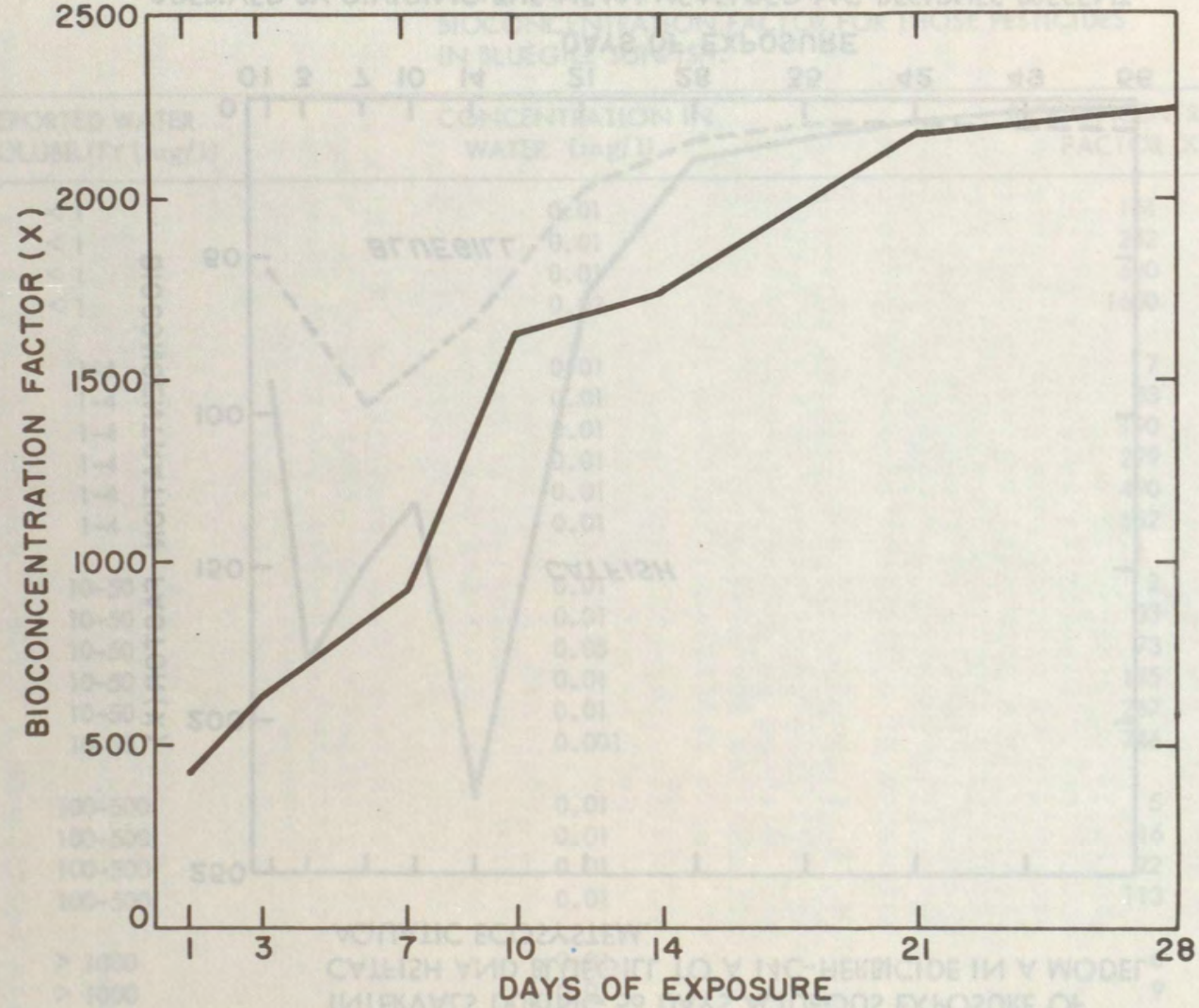
Alternative Methods

We recognize other techniques which may offer alternative methods of assessing the relative propensity of chemicals to concentrate in aquatic organisms. Certainly the use of partition coefficients has received widespread interest. Although we have not utilized partition coefficients, we have attempted to evaluate the degree of correlation between bioconcentration factors and water solubility for the chemicals we have studied. These data (Table 6) suggest an inverse relationship between water solubility and bioconcentration factor such that one may be able to predict the latter from the former within an order of magnitude.

We have recently utilized a model ecosystem approach which more realistically assesses potential hazard for aquatic food chain contamination by pesticides. Utilizing 14C-pesticide,we have "applied" the chemical at recommended use rates utilizing realistic use patterns to soil (or directly to water in a system containing sediment where dictated by recommended or anticipated use patterns). After application of the 14C-pesticide to the system,a reasonable period of "aging" occurs during which the physical, chemical and biological processes which normally occur in natural systems are allowed to effect the chemical residue in the system. After the aging period (2-4 weeks), the aquatic organisms (and water if not already present) are added to the ecosystem and a materials balance study based on radiometric quantitation of 14C-residues in all components is conducted over an additional 6-8 week period.

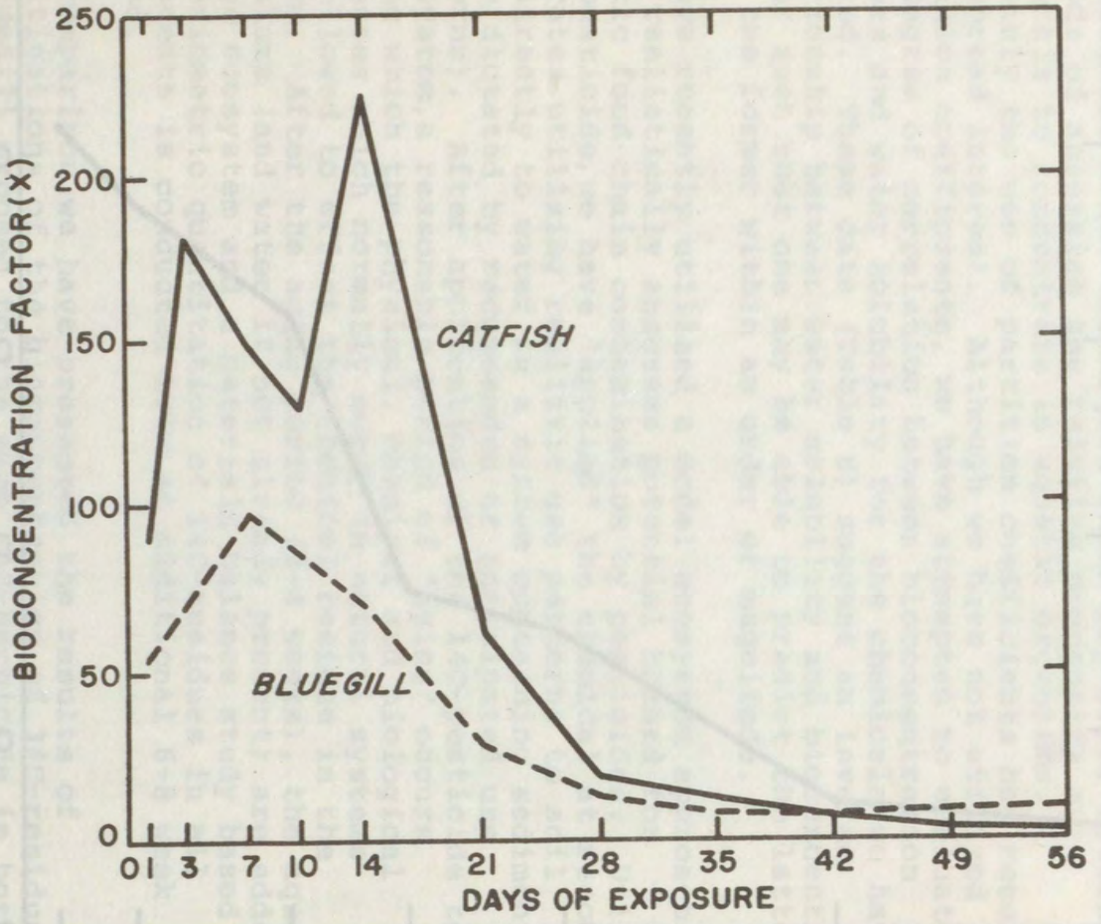
For comparison,we have presented the results of investigations of the bioconcentration of 14C-residues in bluegill exposed to the same 14C-herbicide in both the simple fish-water system (Figure 4) and the model ecosystem (Figure 5). The differences in bioconcentration factors based on 14C-residues in bluegill are indeed dramatic. Gas chromatographic analysis confirmed that this is primarily due to conversion during the aging period in the model system of the parent 14C-herbicide to 14C-degradation products with much lower propensities to accumulate and persist in fish.

FIGURE 4. BIOCONCENTRATION FACTORS* OBSERVED AT VARIOUS INTERVALS DURING 28 DAYS CONTINUOUS EXPOSURE OF BLUEGILL TO 0.001 mg/l 14C-HERBICIDE IN WATER.



*DERIVED BY DIVIDING THE MEAN MEASURED 14C-RESIDUES PRESENT IN FISH (mg/kg) AT ANY POINT IN TIME BY THE MEAN MEASURED 14C-RESIDUES PRESENT IN WATER (mg/l) DURING THE TIME OF EXPOSURE.

FIGURE 5 - BIOCONCENTRATION FACTORS* OBSERVED AT VARIOUS INTERVALS DURING 56 DAYS AQUEOUS EXPOSURE OF CATFISH AND BLUEGILL TO A 14C-HERBICIDE IN A MODEL AQUATIC ECOSYSTEM.



*DERIVED BY DIVIDING THE MEAN MEASURED 14C-RESIDUES PRESENT IN FISH (μg/1) AT ANY POINT IN TIME BY THE MEAN MEASURED 14C-RESIDUES PRESENT IN WATER (μg/1), AT THAT TIME.

TABLE 6 - COMPARISON OF WATER SOLUBILITY (REPORTED) OF PESTICIDE AND THE EMPIRICALLY DETERMINED BIOCONCENTRATION FACTOR FOR THOSE PESTICIDES IN BLUEGILL SUNFISH.

REPORTED WATER SOLUBILITY (mg/l)	CONCENTRATION IN WATER (mg/l)	BIOCONCENTRATION FACTOR (X)
< 1	0.01	161
< 1	0.01	242
< 1	0.01	620
< 1	0.02	1600
1-4	0.01	7
1-4	0.01	83
1-4	0.01	190
1-4	0.01	279
1-4	0.01	490
1-4	0.01	552
10-50	0.01	2
10-50	0.01	33
10-50	0.05	73
10-50	0.01	145
10-50	0.01	267
10-50	0.001	746
100-500	0.01	5
100-500	0.01	16
100-500	0.01	22
100-500	0.01	113
> 1000	0.01	6
> 1000	0.01	9
> 1000	0.01	10

SUMMARY

We have described the results of evaluating the propensity of over 50 radio-labeled pesticides to accumulate in bluegill continuously exposed to chemicals in water for a minimum of 28 days. Bioconcentration factors calculated, and measures of the ability of fishes to depurate the residues upon transfer to uncontaminated water are presented. These data are compared to similar parameters generated for certain chemicals generally considered to pose a hazard to aquatic ecosystems. Clearly, an assessment of the relative propensity of organic chemicals to accumulate in fish offers a potential screening mechanism for identifying those few chemicals which appear to possess properties of accumulation and persistence in aquatic food chains apparently related to the existence of a distinct hazard to these systems.

Alternative methods of assessing relative hazard based on partition coefficients or water solubility are acknowledged as potentially useful. Finally, an alternative for assessing hazard to aquatic ecosystem on a more realistic (i.e. less relative) basis is described.

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A COMPARISON OF METHODS FOR THE ANALYSIS OF THE RELATIONSHIP BETWEEN CHEMICAL AND BIOLOGICAL PROPERTIES OF CHEMICALS

CHAPTER 8

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ABSTRACT

The features, strengths and weaknesses of three methods of data analysis when applied to structure-activity relationships are discussed.

I. GRAPHICAL ANALYSIS

Graphical analysis is an extremely powerful technique for the analysis of the relationships between two or three variables. Not only does it summarize a relationship in a form easily understood by most scientists, but it can reveal causes for concern about the quality of a data set.

In the plot of biological activity vs. a physical property the relationship should not be heavily weighted by one or two points. This type of plot may also suggest what sort of equation might fit the data.

If two properties are predictive of activity one can construct a graph in which the axes are the properties and each observation is identified by its potency. A contour map is thus constructed.

II. REGRESSION ANALYSIS

Reference: Draper and Smith (1966)

Regression analysis is the statistical procedure of determining a least-squares fit of data to some equation. Three statistical parameters are of interest: R^2 measures the fraction of the variance in the data which is explained by an equation; the over-all and partial (each variable) F-values are statistical measures of the probability that a relationship would not occur by chance; and s, the standard error of estimate is a measure of the precision with which the equation predicts the observed values.

There are many aspects to the successful application of regression analysis to a set of data. An important problem when one has several possible predictors is that the total number of possible equations

is 2^P-1 where P is the number of predictor variables. Thus if P=10, there are 1023 possible equations. Therefore, it is often convenient to use step-wise regression techniques in a preliminary look at a data set.

It is important to not evaluate too many possible variables as predictors. Topliss and Costello (1972) reported an empirical study of this problem. They found, for example, that if one has 20 data points, examination of 5 sets of random numbers as predictor variables will result in an R^2 of 0.50, on the average. In this case, on the average only three of the five predictors were statistically significant and included in the calculation of R^2.

Ionizable compounds present another problem. If potency is dependent on the concentration of un-ionized drug present, $(1-\alpha)$, then the log (1/c) value in the correlations should be corrected to the concentration of un-ionized form. (Hansch, 1973; Fujita, 1966) See also the attached figure.

Linear regression analysis is the statistical technique which has been most commonly used in quantitative structure-activity analyses. A linear relationship

$$\log (1/c) = a \log P + b$$

or a parabolic one

$$\log (1/c) = a \log P - b (\log P)^2 + c$$

is easy to calculate. The underlying model is straightforward for the linear case, but not exact for parabolic relationships. To the extent that one wishes to summarize the data at hand a statistical fit can be empirical.

Non-linear regression analysis can be used to fit any equation. We have been interested in drug absorption from the gastrointestinal tract for which the model involves a log P-dependent equilibrium:

$$D_{gut} \rightleftharpoons D_{membrane}$$

and a rate determining step, also log P dependent, out of the membrane into the blood:

$$D_{membrane} \longrightarrow D_{blood}$$

Wagner and Sedman (1973) published equations for this model from which

we were able to show that

$$\log k = b \log P + \log(1-\alpha) - \log [1+dP^{c}(1-\alpha)] + a$$

This equation can describe the case where potency first increases and then decreases (assymmetrically) with increasing log P as well as a biphasic rising relationship or an approach to an asymptote. (Figures appended). Computationally non-linear regression analysis is more complex than linear regression since it is an iterative process and initial estimates of the parameters are necessary.

III. PATTERN RECOGNITION AND DISCRIMINANT ANALYSIS

References: Kowalski and Bender (1974), Overall and Klett (1972), Redl, et al. (1974).

These methods are useful when the biological data is categorical (e.g. active vs. inactive; toxic vs. non-toxic) and there are many associated chemical properties which determine the biological classification. Linear discriminant analysis is a classical multivariate statistical technique (Overall and Klett, 1972). Other pattern recognition methods have developed from computer applications studies, e.g. weather forecasting, handwriting recognition.

IV. SUMMARY

Each of the above methods of data analysis has its own particular features, strengths and weaknesses. The decision of which to use depends on the characteristics of the data, the objectives of the study, and the characteristics of the investigator and his/her facilities.

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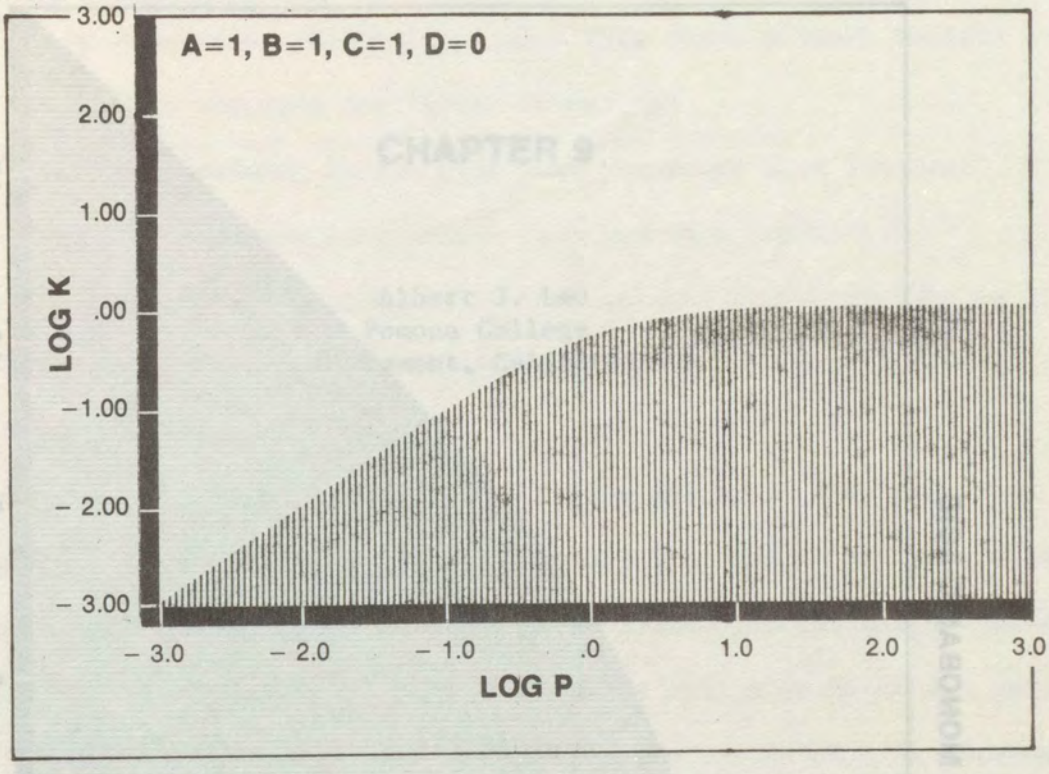
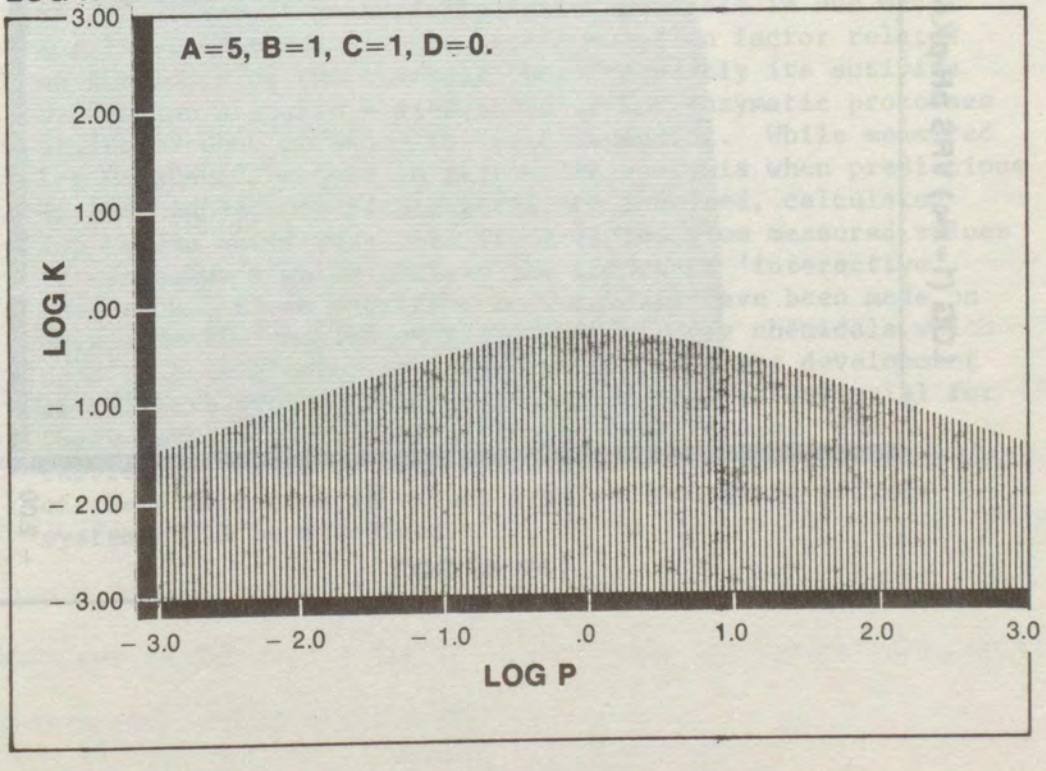
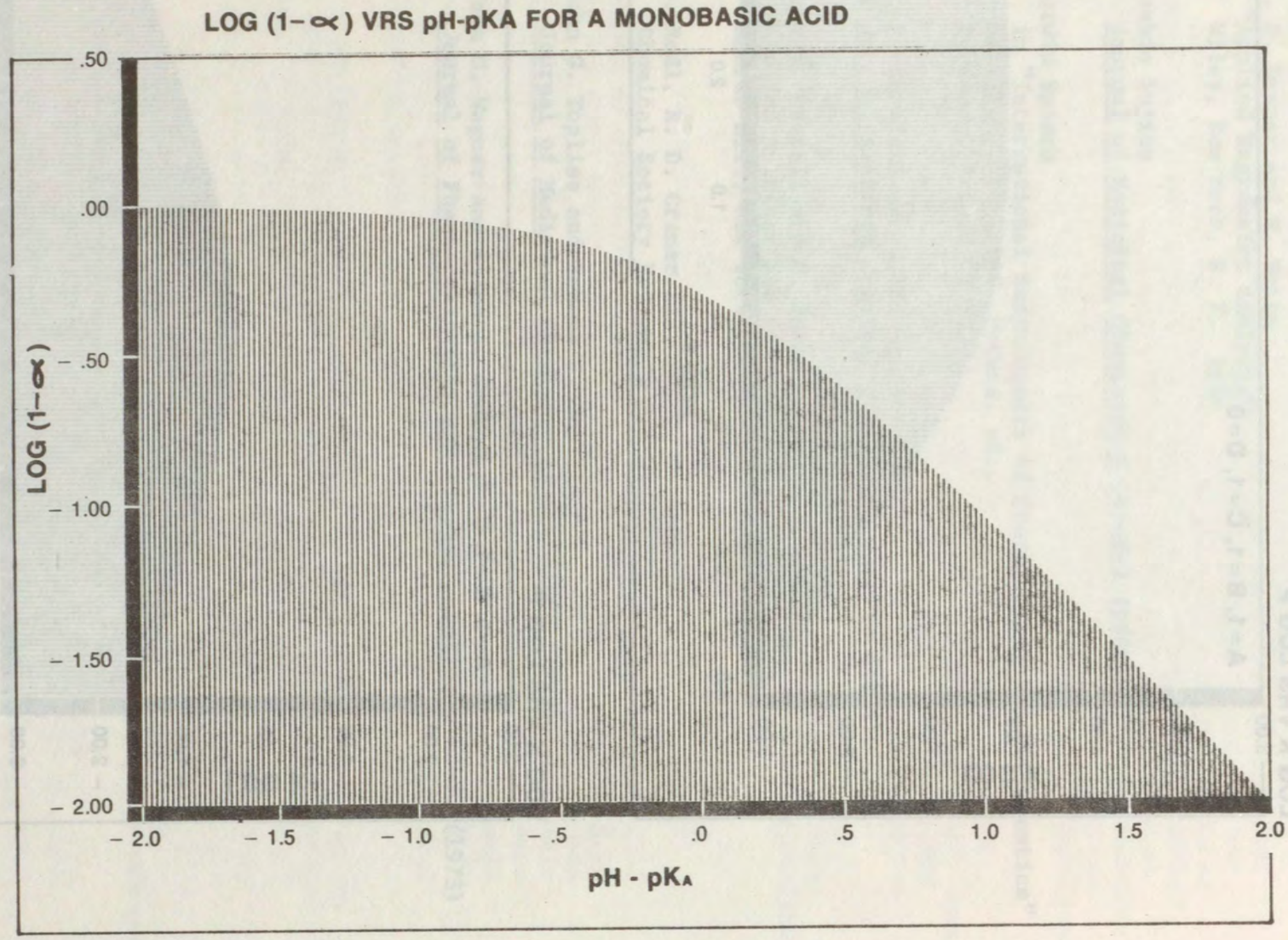
LOG K VRS LOG P



LOG K VRS LOG P




LOG (1−∝) VRS pH-pKA FOR A MONOBASIC ACID
.50
.00
− .50
− 1.00
− 1.50
− 2.00
LOG (1−∝)
− 2.0
− 1.5
− 1.0
− .5
.0
.5
1.0
1.5
2.0
pH - pKA

CALCULATION OF PARTITION COEFFICIENTS USEFUL IN THE EVALUATION OF THE RELATIVE HAZARDS OF VARIOUS CHEMICALS IN THE ENVIRONMENT

CHAPTER 9

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ABSTRACT

The partition coefficient, P, measures the energy involved in transferring molecule of a solute from water to a non-polar solvent and is an essential parameter in the study of the effect of potentially toxic chemicals in our water supplies. Not only is the bioaccumulation factor related on the log P of the chemical, but frequently its activity within the organism - disruption of key enzymatic processes or its metabolic fate - is log P dependent. While measured log Ps should be used in regression analysis when predictions of the highest confidence level are required, calculated log Ps can serve very well if developed from measured values for structures which contain the important 'interactive' fragments. Since partition measurements have been made on only a small fraction of the 10,000 or more chemicals which need to be evaluated as potential hazards, the development of reliable procedures of calculation appears essential for these methods to be applied in the near future. The theoretical basis of such calculations and the significance of the differences in values from various lipid solvent systems will be discussed.

The distribution of a solute between the immiscible solvents, water and octanol, can be treated as though it results from three primary factors (Figure 1):

(1) The energy required for 'hole' formation;

(2) The solute-solvent interaction from permanent bond dipoles;

(3) The solute-solvent interaction from hydrogen bonds.

Of course these three factors operate in both octanol and water, and so it is the sum of the relative effects which determines the equilibrium between phases; that is, the partition coefficient.

Since we are concerned with changes in free energy as they effect an equilibrium, it makes sense to convert the partition coefficient to its log to correspond with the Gibbs expression: $\nabla G = -RT\ln K$. The pi and fragment values which we will be using in our calculations will also be in log terms.

The inert gases constitute the simplest solute system of all, because the solute molecules are spherical (and so the cavity volume is known regardless of how they rotate) and their interaction with the solvent is limited to the short-range forces arising out of electron correlation. In other words, the only factor of any importance is #1.

When the log Ps of the inert gases are plotted against their van der Waals volume, there is the expected general linear relationship (Figure 2), and log P increases with volume because the free energy of cavity formation in water is greater than in octanol. But there are deviations which appear larger than the experimental error especially at the lower volumes. Our data appears to support the postulate that at the lower end of the scale, increasing the size of the cavity in water cannot procede gradually but must occur in discrete steps or quanta. This may be due to the limits of favorable hydrogen-bonding in the water structure surrounding the cavity.

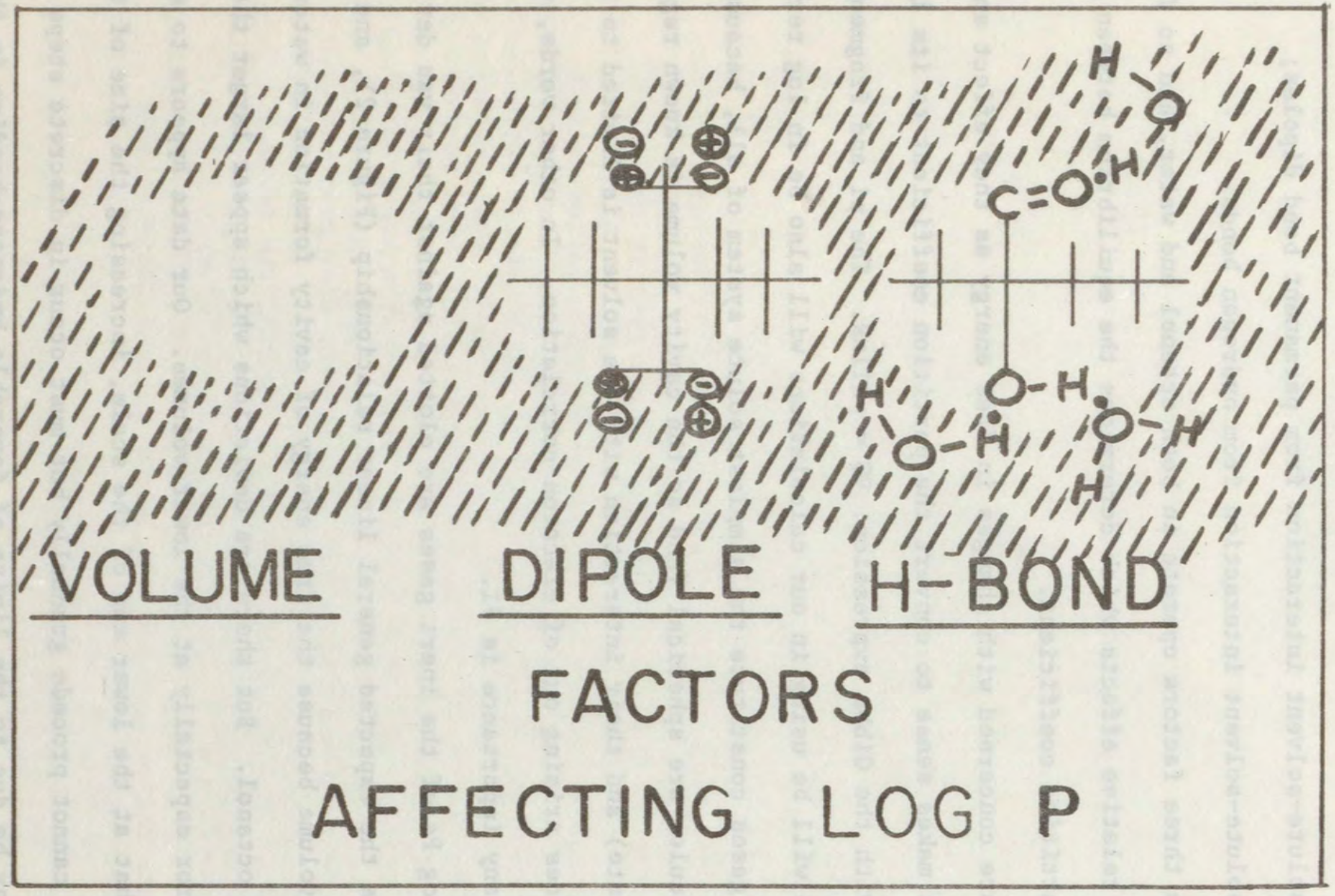


FIGURE 1

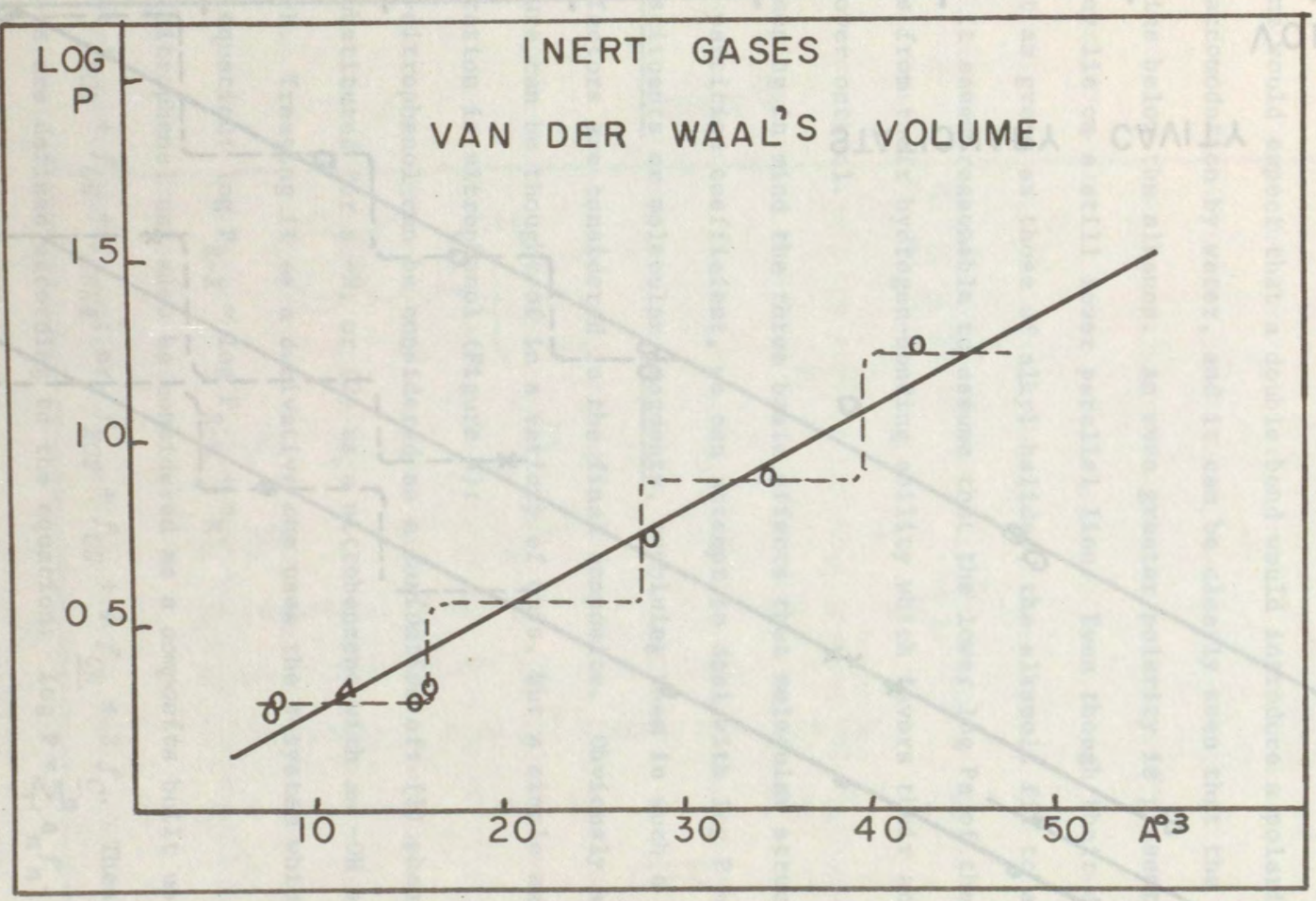


FIGURE 2

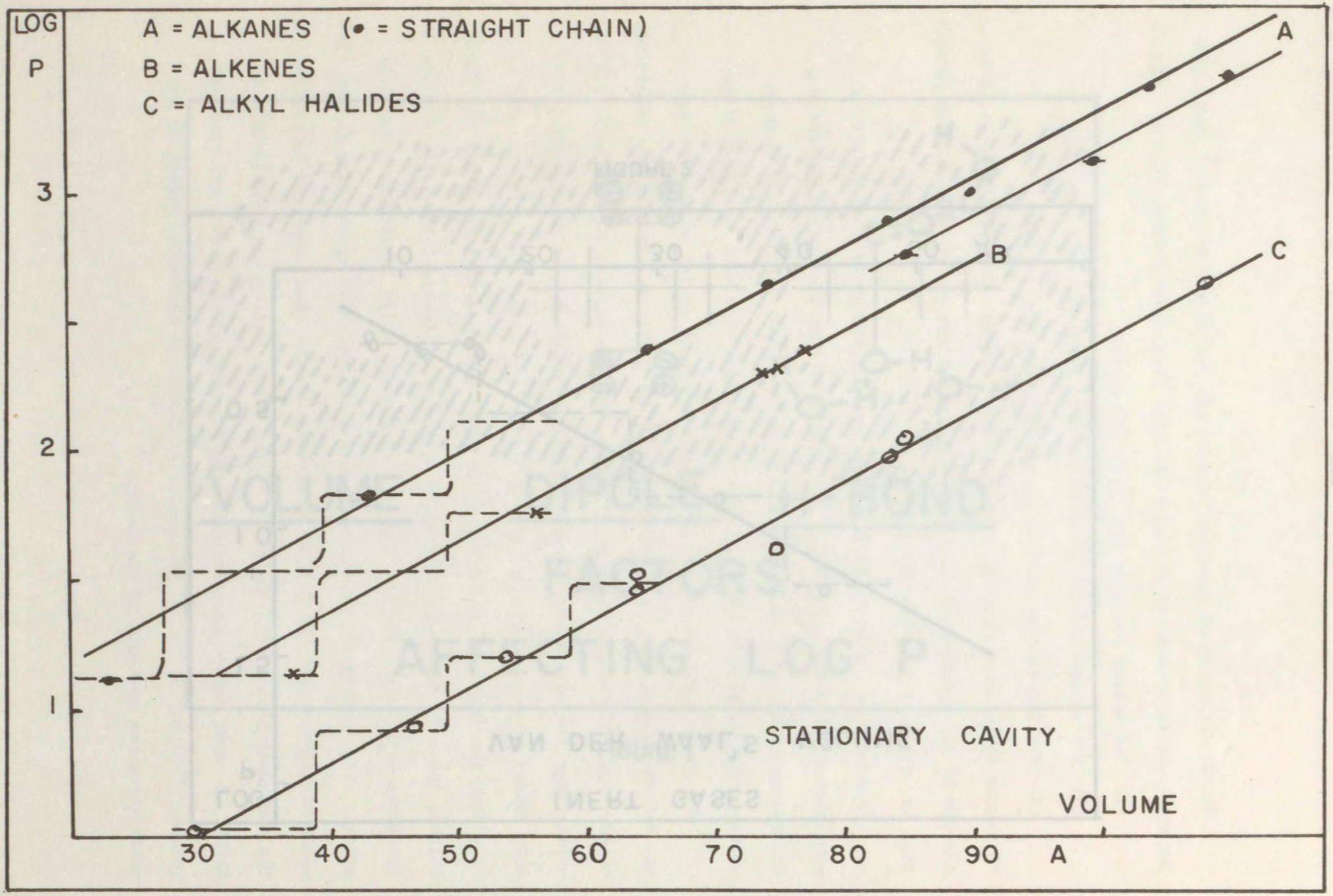

LOG
P
A = ALKANES (• = STRAIGHT CHAIN)
B = ALKENES
C = ALKYL HALIDES
A
B
C
3
2
1
STATIONARY CAVITY
VOLUME
30
40
50
60
70
80
90
A

FIGURE 3

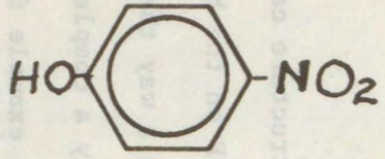
The alkanes show a similar log P vs. volume relationship, but the spherical molecules (methane, neopentane, cyclopentane) are on a line below that for the straight chain homologs (Figure 3).

One would expect that a double bond would introduce a polarity which would favor accomodation by water, and it can be clearly seen that the alkenes lie on a line below the alkanes. An even greater polarity is present in the alkyl halides and they lie on a still lower parallel line. Even though their dipole moments are not as great as those of alkyl halides, the alkanols fit to a still lower line. It seems reasonable to assume that the lower log Ps of the alcohols results from their hydrogen-bonding ability which favors their accomodation by water over octanol.

Keeping in mind the three basic effects that molecular structure can have on the partition coefficient, we can attempt to deal with log P on the basis of substituents or molecular fragments, combining them in such a way that all three factors are considered in the final composite. Obviously a complex structure can be thought of in a variety of ways, but a simple example for illustration is nitrophenol (Figure 4):

p-nitrophenol can be considered as a *derivative* of: (1) phenol with a $-NO_2$ substituted for a -H; or (2) as a nitrobenzene with an -OH substituted for a -H. Treating it as a derivative one uses the π-system which is based on the equation: $\log P_{R-X} = \log P_{R-H} + \pi_X$.

A nitrophenol can also be considered as a *composite* built up of fragments such as: $f_{NO2} + f_{OH} + f_{C6H4}$; or, $f_{NO2} + f_{OH} + 4\, f_{CH} + 2\, f_{C}$. These fragment constants are defined according to the equation: $\log P = \sum_{1}^{n} a_n f_n$.



I. As a Derivative: (use π) $\quad \text{Log } P_{R\text{-}X} = \text{Log } P_{R\text{-}H} + \pi_X$

A. Of phenol with NO_2 substituted for H.

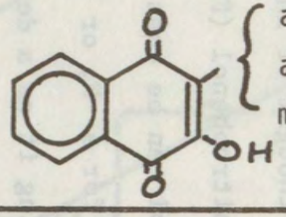
B. Of nitrobenzene, OH substituted for H.

II. As Composite: (use f) $\quad \text{Log P} = \sum_{1}^{n} a_n f_n$

A. $f_{NO_2} + f_{OH} + f_{C_6H_4}$

B. $f_{NO_2} + f_{OH} + 4f_{\underline{CH}} + 2f_{\underline{C}}$

Via I.



alkyl
aryl
mixed

Via II.

aldrin
DDT
$CCl_2 = CCl_2$
etc.

FIGURE 4

The same interplay of the three important partitioning factors is involved whether the 'π-system' or the 'f-system' is used and the choice is a matter of convenience. Generally, if the log P of a rather complex 'parent' is known and the log Ps of a series of derivatives must be calculated, the pi-system is preferrable, because any interaction terms present in the parent (such as conjugated carbonyls and the o-OH in the example) are already accounted for. But in trying to arrive at close estimates for a variety of chemicals such as those which have either been found in water supplies or thought likely to enter them, the fragment approach will be more suitable.

So I will devote the rest of the time to show how it is possible to combine fragment values with the proper interaction terms to derive reasonably close estimates of the log P values of solutes whose measured values may be difficult or impossible to come by.

In the development of a series of hydrophobic fragment constants, our group at Pomona began with careful measurements of the simple, non-polar solutes where the cavity-volume factor predominates in determining the log P. (The Nys-Rekker group in Holland used a statistical approach employing our computerized data base to originate the 'fragment constant' concept. It would take too much time to discuss the pros and cons of each approach, but we feel the needs of the workers in the structure-activity field will be best served if <u>both</u> methods are fully explored.)

For the aliphatic series, we felt there were three partition coefficients of primary significance:

(1) $\log P_{H_2} = 0.45$

(2) $\log P_{CH_4} = 1.09$

(3) $\log P_{CH_3CH_3} = 1.81$

From these we can calculate: $f_H = 0.225$

$$f_{CH_3} = f_{CH_4} - f_H = 0.865; \text{ or,}$$
$$f_{CH_3} = \tfrac{1}{2}\log P_{CH_3CH_3} = 0.905 \quad \Big\} \text{ Average} = 0.885$$

As important as they are, these Fundamental Constants are not, in themselves, a proper foundation for a computation scheme. In our view successful computation of log Ps depended on the introduction of a 'flexibility factor' which apparently reflects the ability of certain structures to assume lower energy conformations in aqueous solution. This 'inflexibility' is taken into account by means of bond and branching constants. When log P is plotted against chain length for the normal alkanes (Figure 5), we see that the value per methylene group levels off at 0.54, but the first two members require special treatment. An effective way to handle this is to assign a negative fragment value of -.12 to each fragment-to-fragment bond *after the first one* in the solute structure. After rounding off and averaging the 'fundamental values', we get:

$f_H = .23$
$f_{CH_3} = .89$
$f_{CH_2} = .66 = f_{CH_3} - .23$
$f_{CH} = .43 = f_{CH_2} - .23$
$f_C = .20 = f_{CH} - .23$

$f_b = -.12$
$f_{\underline{b}} = -.09$
$f_{cbr} = -.13$
$f_{gbr} = -.22$

Although this partitioning evidence is obviously not sufficient support in itself, it is convenient to consider the 'bond fragment' constants as a volume reduction factor resulting from added flexibility. The bonds which form a ring would not be as effective in this regard, and it is clear that better agreement is obtained when $f_{\underline{b}}$ is assigned a value of -.09 rather than -.12.

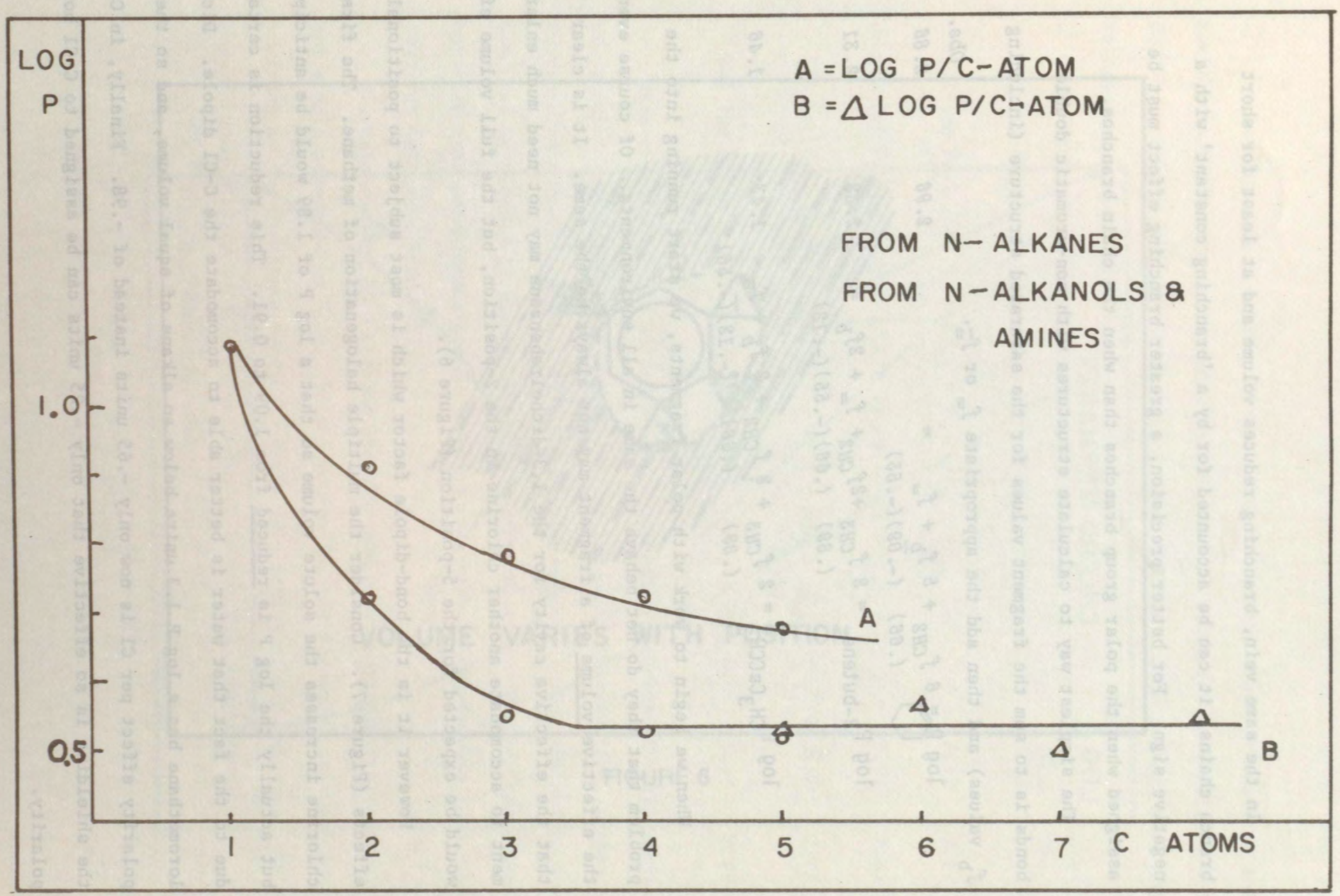

LOG
P
A = LOG P/C-ATOM
B = Δ LOG P/C-ATOM
FROM N-ALKANES
FROM N-ALKANOLS &
AMINES
1.0
0.5
A
B
1
2
3
4
5
6
7
C ATOMS

FIGURE 5

In the same vein, branching reduces volume and at least for short branch chains, it can be accounted for by a 'branching constant' with a negative sign. For better precision, a greater branching effect must be assigned when the polar group branches than when the chain branches.

The simplest way to calculate structures with non-aromatic double bonds is to sum the fragment values for the saturated structure (including f_b values) and then add the appropriate $f_=$ or $f_{\overline{=}}$.

		Obs.
log P  $= 6\,f_{CH2} + 5\,f_b + f_=$ (.66) (-.09)(-.55)	2.96	2.86
log $P_{2\text{-butene}}$ $= 2\,f_{CH3} + 2f_{CH2} + f_= + 2f_b$ (.89) (.66)(-.55)(-.12)	2.32	2.31
log $P_{CH_3C\equiv CCH_3}$ $= 2\,f_{CH3} + 2\,f_{CH2} + 2\,f_b + f_{\equiv}$ (.89) (.66) (-.12)(-.55)	1.43	1.46

When we begin to work with polar fragments, we start running into the problem that they do not behave the same in all environments. Of course even the effective volume of a fragment may not always be the same. It is clear that the effective cavity for the 1,3-dicholrobenzene may not need much enlargement to accomodate another chlorine in the 2-position, but the full volume effect would be expected for the 5-position (Figure 6).

However it is the bond-dipole factor which is most subject to positional effects (Figure 7). Consider the multiple halogenation of methane. The first chlorine increases the solute volume so that a log P of 1.89 would be anticipated, but actually the log P is reduced from 1.09 to 0.91. This reduction is certainly due to the fact that water is better able to accomodate the C-Cl dipole. Dichloromethane has a log P 1.3 units below an alkane of equal volume, and so the polarity effect per Cl is now only -.65 units instead of -.98. Finally, in CCl_4 the shielding is so effective that only -.25 units can be assigned to C-Cl bond polarity.

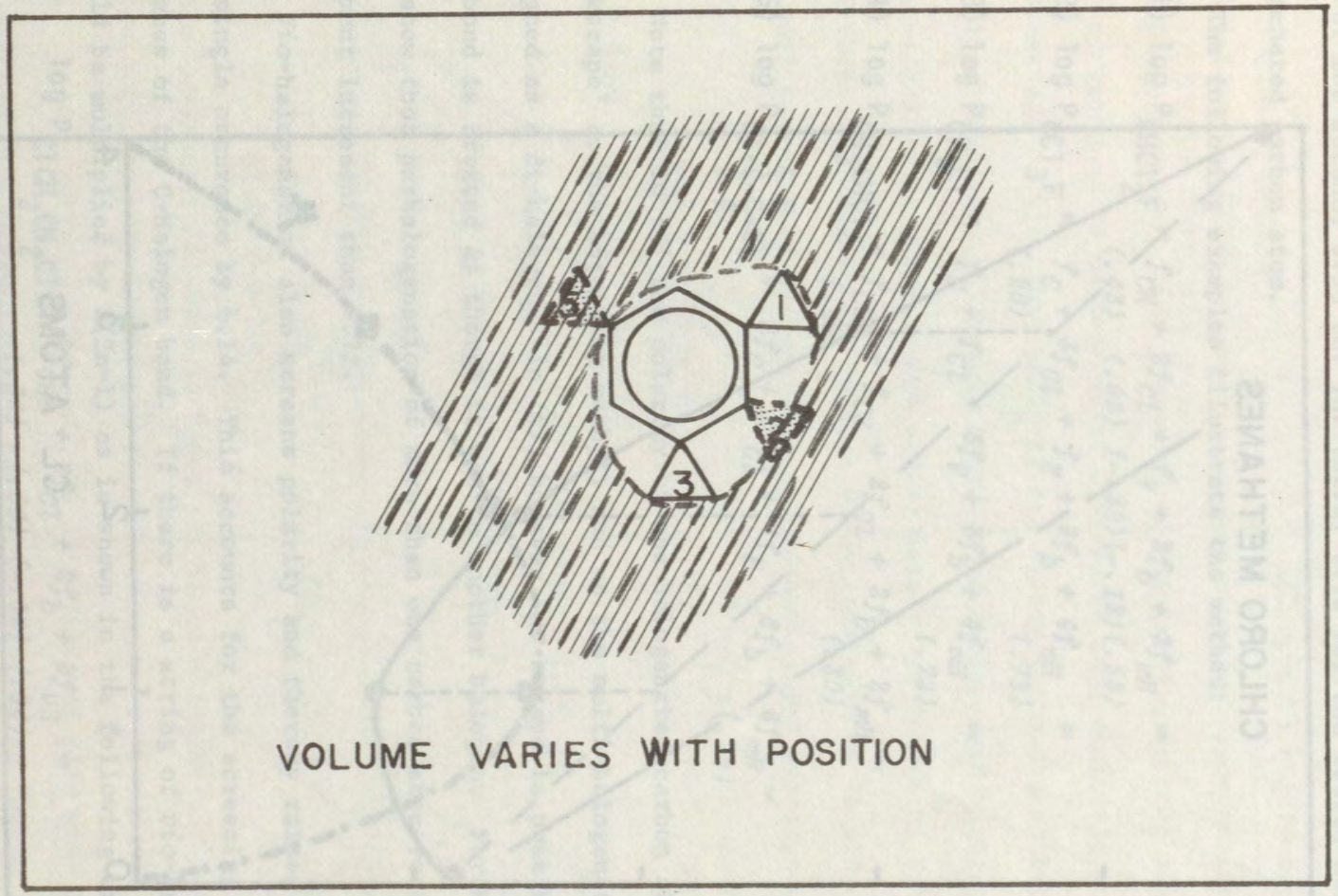


FIGURE 6

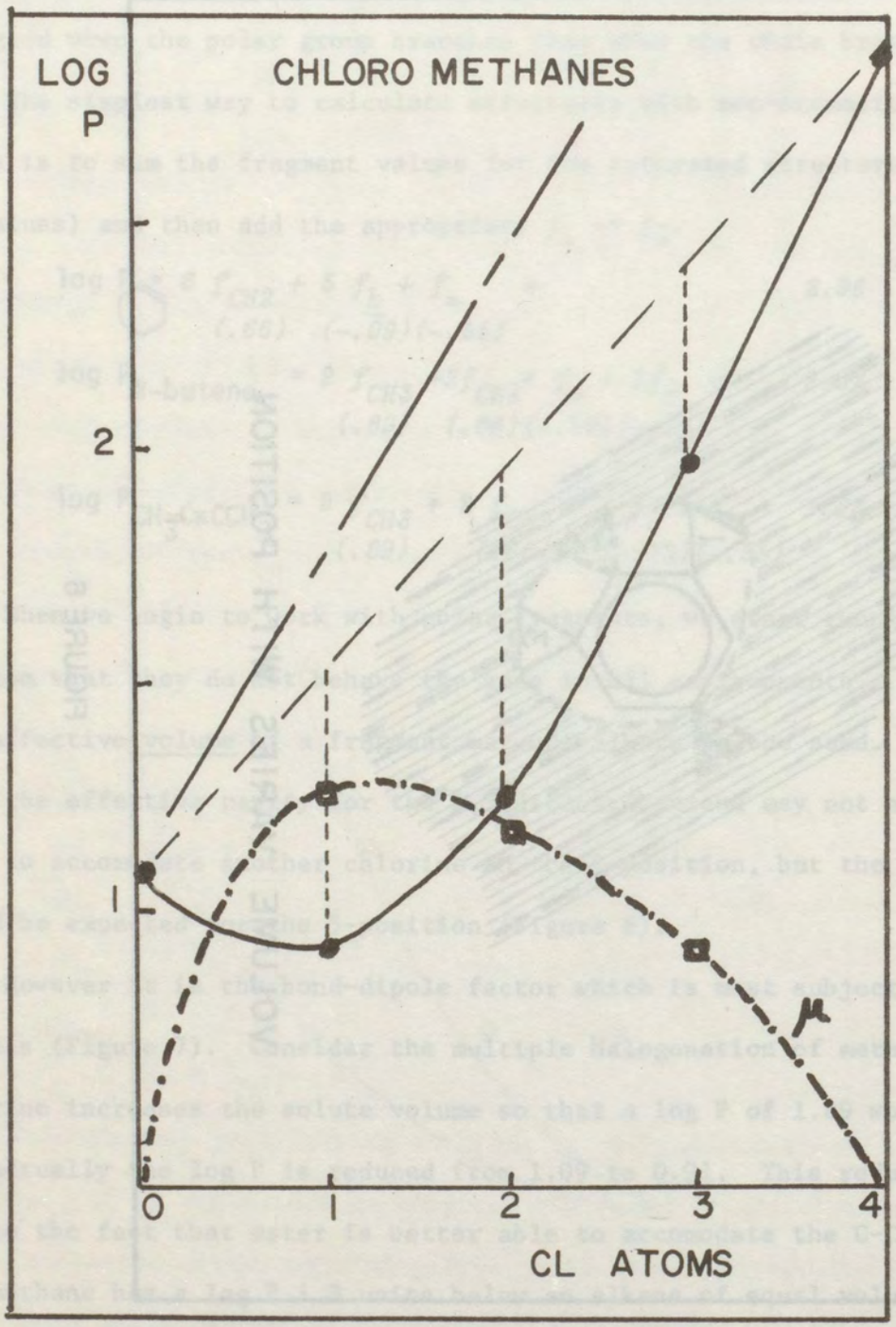


FIGURE 7

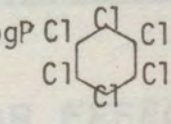
The calculation procedure we use is to assign the fragment value on the basis of mono-substitution, and then increase the value for *each* halogen by: 0.3 for the second; 0.53 for the third; 0.72 for the fourth halogen on a multi-halogenated carbon atom.

The following examples illustrate the method:

		Calc.	Obs.
(1)	$\log P_{CHCl_2F} = \underset{(.43)}{f_{CH}} + \underset{(.06)}{2f_{Cl}} + \underset{(-.38)}{f_F} + \underset{(-.12)}{2f_b} + \underset{(.53)}{3f_{mH}} =$	1.52	1.55
(2)	$\log P_{CCl_3F} = \underset{(.20)}{f_C} + 3f_{Cl} + f_F + 3f_b + \underset{(.72)}{4f_{mH}} =$	2.52	2.53
(3)	$\log P_{CF_3Cl} = f_C + f_{Cl} + 3f_F + 3f_b + \underset{(.72)}{4f_{mH}} =$	1.64	1.65
(4)	$\log P_{CH_3CHCl_2} = \underset{(.89)}{f_{CH3}} + f_{CH} + 2f_{Cl} + 2f_b + \underset{(.30)}{2f_{mH}} =$	1.80	1.79
(5)	$\log P_{Cl\text{-}\overset{F}{\overset{|}{C}}\text{-}\overset{F}{\overset{|}{C}}\text{-}Cl} = 2f_C + 2f_{Cl} + 4f_F + 6f_b + \underset{(.72)}{6f_{mH}} =$	2.60	2.82

Note that in *(4)* the polarity of the halogenated carbon atom has a means of 'escape' to the adjoining methyl, and so the multi-halogenation fragment is assigned as a di-halogen. In *(5)*, however, no escape is possible and so the C-C bond is treated as though it was to another halogen. Further measurements may show that perhalogenation of more than one carbon atom results in a higher fragment increment than +.72.

Vic-halogenation also screens polarity and thereby raises the fragment value per single occurence by +.14. This accounts for the screening on the 'inner' surfaces of the C-Halogen bond. If there is a string of *vic*-halogens, the effect should be multiplied by 2(2n-1) as is shown in the following examples:

$\log P_{ClCH_2CH_2Cl}$	$= \underset{(.66)}{2f_{CH2}} + \underset{(.06)}{2f_{Cl}} + \underset{(-.12)}{2f_b} + \underset{(.14)}{2f_{vH}} =$	1.48	1.48
$\log P$ 	$= \underset{(.43)}{6f_{CH}} + \underset{(.06)}{6f_{Cl}} + \underset{(-.09)}{5f_b} + \underset{(.14)}{2(n-1)f_{vH}} =$	4.03	3.72 to 4.14

In all the previous examples, the carbon-halogen dipole itself was responsible for the reduction in hydrophobicity. If the polarity affects a hydrogen-bonding functional group, the effect can be very great and it usually increases hydrophobicity (Figure 8).

We have not, as yet, devised a workable 'fragment-interaction' factor to take care of this type of effect, and we recommend that these calculations be approached using pi-values from as close a derivative as it is possible to find.

When two hydrogen-bonding groups are in close proximity to one another part of their hydrophilic character is lost. This H-bond 'proximity effect' also raises log P, but it cannot be treated in the same fashion as multiple- and *vic*-halogenation. This is an area that needs a great deal of further study to develop more reliable constants, but reasonable good values can be expected from these interaction terms:

CHAIN

$O\text{-}C_n\text{-}O$

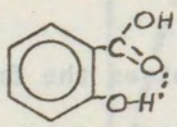
$HO\text{-}C_n\text{-}OH$

$HO_2C\text{-}C_n\text{-}OH$ $\quad f_{px1}$=+.8 (where n=1)

$HO\text{-}C_n\text{-}NH\text{-}$ $\quad f_{px2}$=+.4 (where n=2)

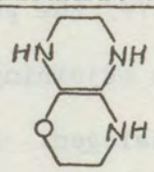
$-C^{O}\text{-}C_n\text{-}CO_2H$

INTRA-MOL. H-BOND



f_{Hb} = +.70

ALIPHATIC RING



each $f_{\underline{px2}}$ =.42$_5$

each $f_{\underline{px2}}$ =.32$_5$

"AROMATIC'RING

$f^{\phi}_{pxN\text{-}NH}$ = 1.19

$f^{\phi}_{px1\text{-}N\text{-}NH}$=0.58

$f^{\phi}_{px1\text{-}N\text{-}N}$ =0.42

$f^{\phi}_{px2\text{-}N\text{-}N}$ =0.32

To carry out the calculation of the log P of aromatic compounds we need another set of 'enhanced' fragment constants for all of the polar groups. To avoid confusing them with the aliphatic set, we use a super-script as: f^{ϕ}. Those fragments which can be attached to two aromatic rings are doubly enhanced and are designated as: $f^{\phi\phi}$.

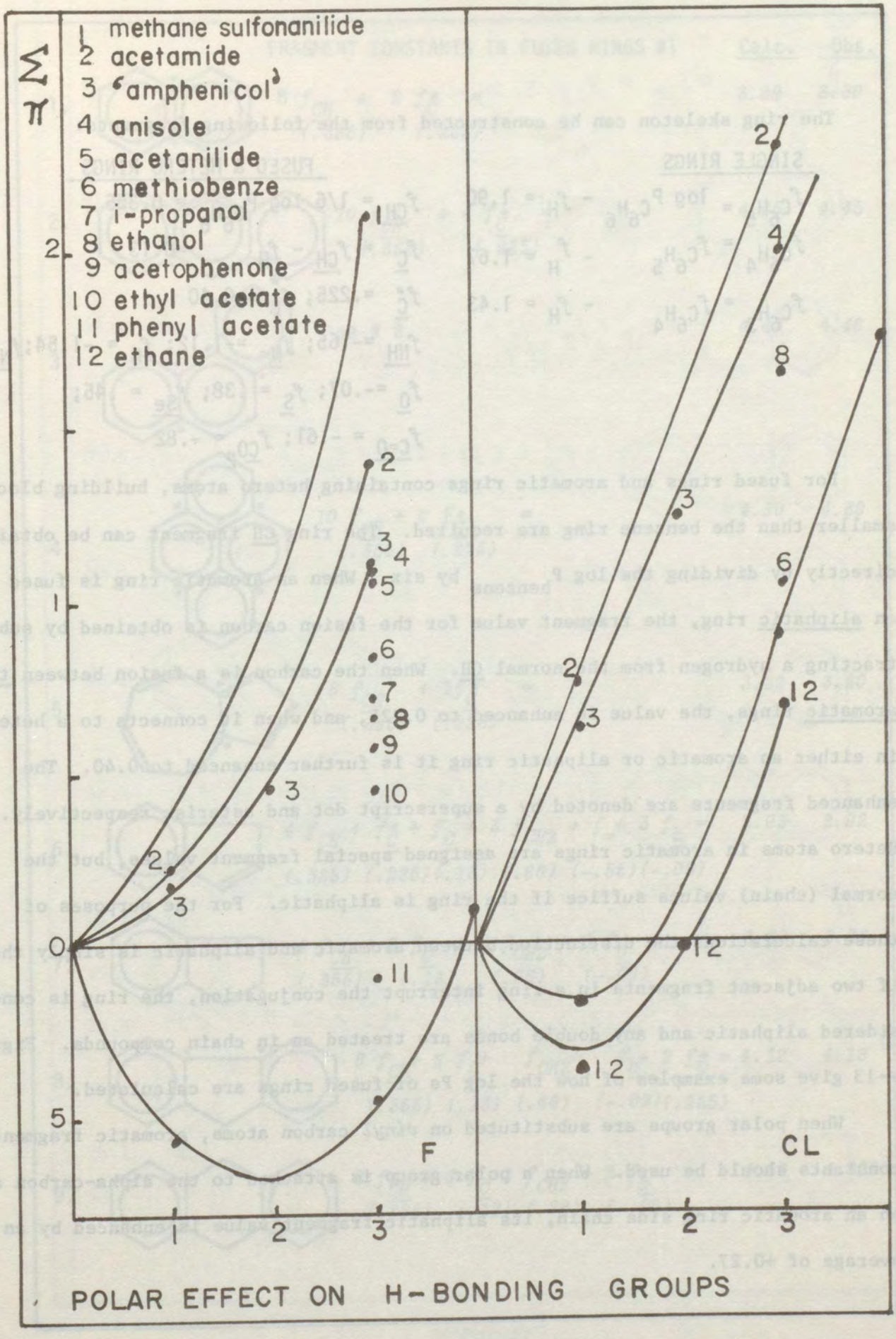

Σπ
1 methane sulfonanilide
2 acetamide
3 'amphenicol'
4 anisole
5 acetanilide
6 methiobenze
7 i-propanol
8 ethanol
9 acetophenone
10 ethyl acetate
11 phenyl acetate
12 ethane
2
1
0
5
F
CL
1
2
3
POLAR EFFECT ON H-BONDING GROUPS

FIGURE 8

The ring skeleton can be constructed from the following fragments:

SINGLE RINGS	FUSED & HETERO RINGS
$f_{C_6H_5} = \log P_{C_6H_6} - f_H = 1.90$	$f_{\underline{CH}} = 1/6 \log P_{C_6H_6} = 0.355$
$f_{C_6H_4} = f_{C_6H_5} - f_H = 1.67$	$f_{\underline{C}} = f_{\underline{CH}} - f_H = 0.13$
$f_{C_6H_3} = f_{C_6H_4} - f_H = 1.43$	$f_{\underline{C}}^{\cdot} = .225$; $f_{\underline{C}}^{*} = 0.40$
	$f_{\underline{NH}} = -.65$; $f_{\underline{N=}} = -1.12$; $f_{\underline{N}} = -1.54$; $f_{\underline{N}}^{+} = -6.31$
	$f_{\underline{O}} = -.07$; $f_{\underline{S}} = .38$; $f_{\underline{Se}} = .45$;
	$f_{\underline{C=O}} = -.61$; $f_{\underline{CO_2}} = -.82$

For fused rings and aromatic rings containing hetero atoms, building blocks smaller than the benzene ring are required. The ring CH fragment can be obtained directly by dividing the $\log P_{benzene}$ by six. When an aromatic ring is fused to an aliphatic ring, the fragment value for the fusion carbon is obtained by subtracting a hydrogen from the normal CH. When the carbon is a fusion between two aromatic rings, the value is enhanced to 0.225, and when it connects to a heteroatom in either an aromatic or aliphatic ring it is further enhanced to 0.40. The enhanced fragments are denoted by a superscript dot and asterisk respectively. Hetero atoms in aromatic rings are assigned special fragment values, but the normal (chain) values suffice if the ring is aliphatic. For the purposes of these calculations the distinction between aromatic and aliphatic is simply that if two adjacent fragments in a ring interrupt the conjugation, the ring is considered aliphatic and any double bonds are treated as in chain compounds. Figures 9-13 give some examples of how the log Ps of fused rings are calculated.

When polar groups are substituted on *vinyl* carbon atoms, aromatic fragment constants should be used. When a polar group is attached to the alpha-carbon atom on an aromatic ring side chain, its aliphatic fragment value is enhanced by an average of +0.27.

FRAGMENT CONSTANTS IN FUSED RINGS #1

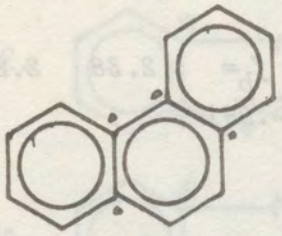
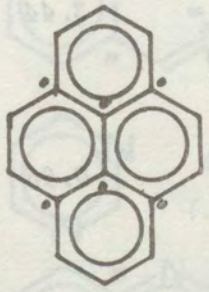
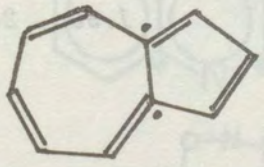
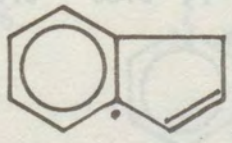
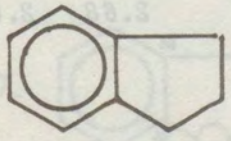
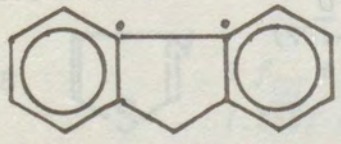
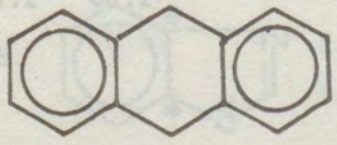
			Calc.	Obs.
1.		$8\,f_{\underline{CH}}\,(.355) + 2\,f_{\underline{\dot{C}}}\,(.225) =$	3.29	3.30
2.		$10\,f_{\underline{CH}}\,(.355) + 4\,f_{\underline{\dot{C}}}\,(.225) =$	4.45	4.45
3.		as # 2	4.45	4.46
4.		$10\,f_{\underline{CH}}\,(.355) + 6\,f_{\underline{\dot{C}}}\,(.225) =$	4.90	4.88
5.		$8\,f_{\underline{CH}}\,(.355) + 2f_{\underline{\dot{C}}}\,(.225) =$	3.29	3.20
6.		$4\,f_{\underline{CH}}\,(.355) + f_{\underline{\dot{C}}}\,(.225) + f_{\underline{C}}\,(.13) + 3\,f_{CH2}\,(.66) + f_{=}\,(-.55) + 3\,f_{\underline{b}}\,(-.09) =$	2.93	2.92
7.		$4\,f_{\underline{CH}}\,(.355) + 2\,f_{\underline{C}}\,(.13) + 3\,f_{CH2}\,(.66) + 3\,f_{\underline{b}}\,(-.09) =$	3.39	3.33
8.		$8\,f_{\underline{CH}}\,(.355) + 2\,f_{\underline{C}}\,(.13) + f_{CH2}\,(.66) + f_{\underline{b}}\,(-.09) + 2\,f_{\underline{\dot{C}}}\,(.225) =$	4.12	4.18
9.		$8\,f_{\underline{CH}}\,(.355) + 4\,f_{\underline{C}}\,(.13) + 2\,f_{CH2}\,(.66) + 2\,f_{\underline{b}}\,(-.09) =$	4.50	4.29?

FIGURE 9

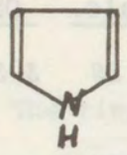
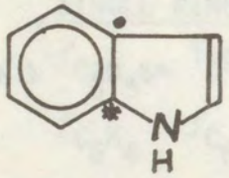
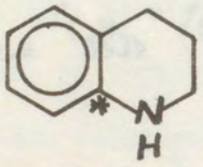
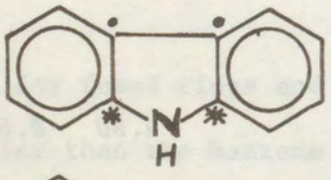
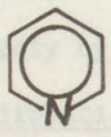
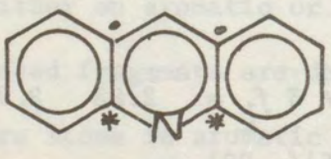
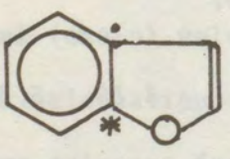
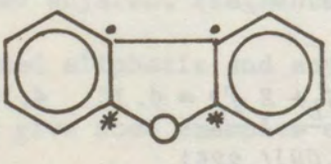
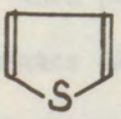
FRAGMENT CONSTANTS IN FUSED RINGS #2		Calc.	Obs.
10. 	$4\,f_{\underline{CH}} + f_{\underline{NH}} =$ $(.355)\ \ (-.65)$	0.77	0.75
11. 	$6\,f_{\underline{CH}} + f_{\underline{C}}^{\bullet} + f_{\underline{C}}^{*} + f_{\underline{NH}} =$ $(.355)\ (.225)\ (.40)\ (-.65)$	2.11	2.14
12. 	$4\,f_{\underline{CH}} + f_{\underline{C}} + f_{\underline{C}}^{*} + f_{NH}^{\phi} + 3\,f_{CH2} + 4\,f_{\underline{b}} =$ $(.355)\ (.13)\ (.40)\ (-1.03)\ (.66)\ (-.09)$	2.38	2.29
13. 	$8\,f_{\underline{CH}} + 2\,f_{\underline{C}}^{\bullet} + 2\,f_{\underline{C}}^{*} + f_{\underline{NH}} =$ $(.355)\ \ (.225)\ \ (.40)\ (-.65)$	3.44	3.72 3.29
14. 	$5\,f_{\underline{CH}} + f_{\underline{N=}} =$ $(.355)\ (-1.12)$	0.65	0.65
15. 	$7\,f_{\underline{CH}} + f_{\underline{C}}^{\bullet} + f_{\underline{C}}^{*} + f_{\underline{N=}} =$	1.99	2.03
16. 	$9\,f_{\underline{CH}} + 2\,f_{\underline{C}}^{\bullet} + 2\,f_{\underline{C}}^{*} + f_{\underline{N=}} =$ $(.355)\ (.225)\ \ (.40)\ (-1.12)$	3.30	3.40
17. 	$6\,f_{\underline{CH}} + f_{\underline{C}}^{\bullet} + f_{\underline{C}}^{*} + f_{\underline{O}} =$ $(.355)\ (.225)\ (.40)\ (-.07)$	2.68	2.67
18. 	$8\,f_{\underline{CH}} + 2\,f_{\underline{C}}^{\bullet} + 2\,f_{\underline{C}}^{*} + f_{\underline{O}} =$ $(.355)\ \ (.225)\ \ (.40)\ (-.07)$	4.02	4.12
19. 	$4\,f_{\underline{CH}} + f_{\underline{S}} =$ $(.355)\ \ (.38)$	1.80	1.81

FIGURE 10

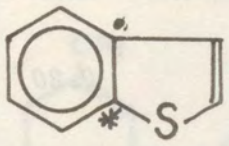
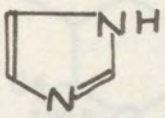
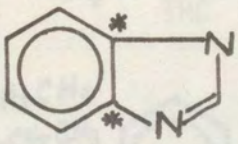
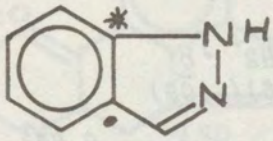
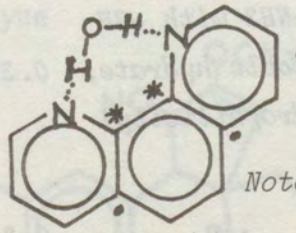
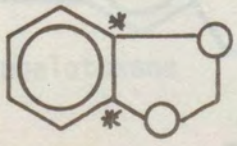
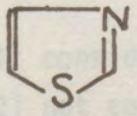
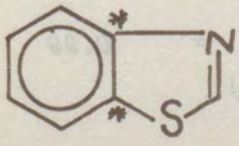
FRAGMENT CONSTANTS IN FUSED RINGS #3		Calc.	Obs.
20.	 $6\,f_{CH} + f_{\dot{C}} + f_{C}^{*} + f_{S} =$ $(.355)(.225)\ (.40)\ (.38)$	3.13	3.12
21.	 $3\,f_{CH} + f_{NH} + f_{N=} + f_{pe1} =$ $(.355)\ (-.65)\ (-1.12)\ (.58)$	-.12	-.08
22.	 $5\,f_{CH} + 2\,f_{C}^{*} + f_{NH} + f_{N=} + f_{pe1} =$ $(.355)\ (.40)\ (-.65)\ (-1.12)\ (.58)$	1.38	1.34
23.	 as #22 $+ f_{pe} =$ (1.19)	1.82	1.82
24.	 $4\,f_{CH} + 2\,f_{N=} + f_{pe1} =$ $(.355)\ (-1.12)\ (.42)$	-.40	-.40
25.	 $8\,f_{CH} + 4\,f_{C}^{*} + 2\,f_{N=} + 2\,f_{pe2} =$ $(.355)\ (.40)\ (-1.12)\ (.32)$	2.84	2.84
26.	 $8\,f_{CH} + 2\,f_{\dot{C}} + 2\,f_{C}^{*}\ \ 2\,f_{N=}$ $+ f_{pe2} =$ Note: Hydrate may offset proximity effect.	2.17	1.85
27.	 $4\,f_{CH} + 2\,f_{C}^{*} + f_{CH2} + 2\,f_{O}^{\phi} + 3\,f_{b} +$ $(.355)\ (.40)\ (.66)\ (-.57)\ (-.09)$ $+ f_{pe1} =$ $(.78)$	2.08	2.08
28.	 $3\,f_{CH} + f_{N=} + f_{S} + f_{pe1} =$ $(.355)\ (-1.12)\ (.38)\ (.15)$	0.47	0.44
29.	 $5\,f_{CH} + 2\,f_{C}^{*} + f_{N=} + f_{S} + f_{pe1} =$ $(.355)\ (.40)\ (-1.12)\ (.38)\ (.15)$	1.99	2.01

FIGURE 11

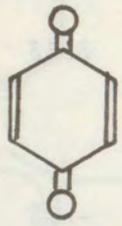
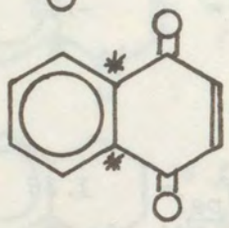
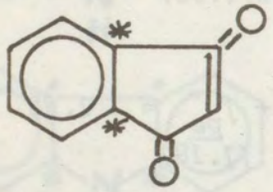
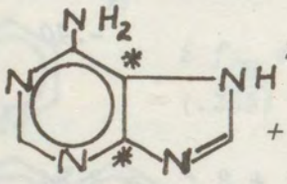
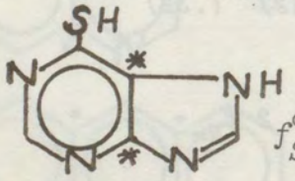
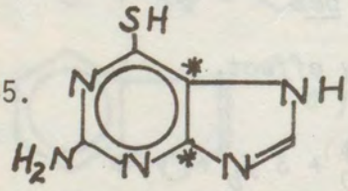
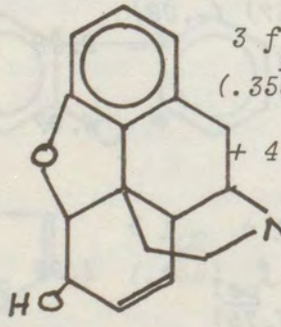
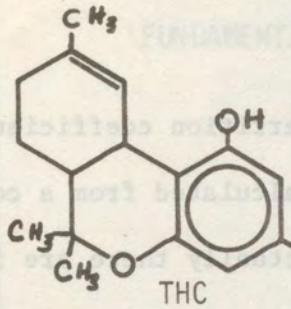
FRAGMENT CONSTANTS IN FUSED RINGS #4		Calc.	Obs.
30.	 $4\ f_{\underline{CH}} + 2\ f_{\underline{C=O}} =$ (.355) (-.61)	0.20	0.20
31.	 $6\ f_{\underline{CH}} + 2\ f^{*}_{\underline{C}} + 2\ f_{\underline{C=O}} =$ (.355) (.40) (-.61)	1.71	1.71 1.78
32.	 $4\ f_{\underline{CH}} + 2\ f^{*}_{\underline{C}} + 2\ f^{\phi}_{C=O} + f_{CH2} + f_{\underline{b}}$ (.355) (.40) (-1.09) (.66) (-.09) $+ f_{\underline{pe1}} =$ (.18)	0.61	0.61
33.	 $2\ f_{\underline{CH}} + f_{\underline{C}} + 2\ f^{*}_{\underline{C}} + f_{\underline{NH}} + 3\ f_{\underline{N=}}$ $+ f^{\phi}_{NH2}{}^{*} + 2\ f_{\underline{peNN1}} + f_{\underline{peNM1}} + 2 f_{\underline{peNN2}}$ (.15) (.50) (.58) (.25)	-.14	-.09
	*exalted -NH2 as in 0-nitroaniline		
34.	 as #33 replacing -NH2 with -SH $f^{\phi}_{SH} = 0.62$ (forms stable hydrate; may explain hydrophilicity)	0.33	0.01
35.	 #34 $-f_{H} + f^{\phi}_{NH2}{}^{*} =$	0.25	-0.06
36.	 $3\ f_{\underline{CH}} + 3\ f_{\underline{C}} + f^{\phi}_{O} + 5\ f_{CH2} + f_{\underline{=}}$ (.355) (.13) (-.57) (.66) (-.55) $+ 4\ f_{CH} + f_{C} + f_{N} + f_{CH3} + f_{b}$ (.43) (.20) (-2.16) (.89) (-.12) $+ 15\ f_{\underline{b}} + f_{OH} + f_{br} =$ (-.09) (-1.63) (-.20)	0.99	1.03

FIGURE 12

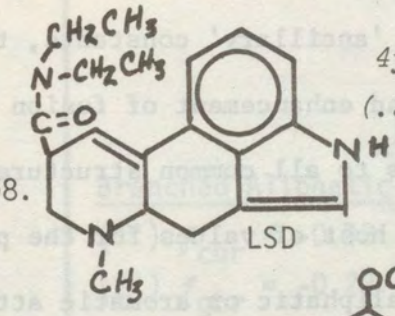
FRAGMENT CONSTANTS IN FUSED RINGS #5

Calc. Obs.

37. 

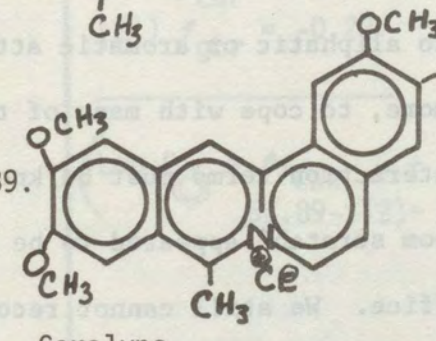
THC

$2f_{CH} + 3f_{C} + f^{*}_{C} + f_{C} + 3f_{CH} + 7f_{CH2}$
(.355) (.13) (.40)(.20) (.43) (.66)
$+ 4f_{CH3} + f^{\phi}_{OH} + f^{\phi}_{O} + 9f_{b} + 7f_{b} +$
(.89) (-.40)(-.57)(-.09)(-.12)
$+ 3f_{cbr} + f_{=}$ = 7.61 3.8
(-.13) (-.55)

38. 

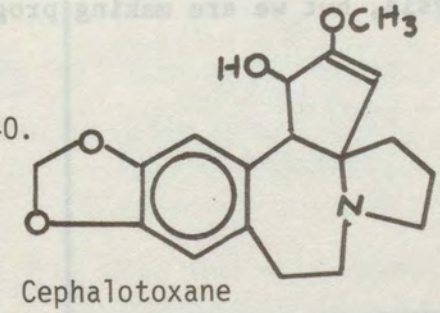
LSD

$4f_{CH} + f^{*}_{C} + f^{\cdot}_{C} + 2f_{C} + 5f_{CH2} + f_{=}$
(.355) (.40)(.225)(.13) (.66)(-.55)
$+ 3f_{CH} + 3f_{CH3} + f_{NH} + f_{N}$
(.43) (.89) (-.65)(-2.16)
$+f_{CON} + 8f_{b} + 5f_{b}$ = 1.69 2.98

39. 

Coralyne

$7f_{CH} + 5f_{C} + 5f^{\cdot}_{C} + 5f_{CH3}$
(.355)(.13) (.225) (.89)
$+ 4f^{\phi}_{O} + 4f_{b} + f_{N+}$ = -.36 -.53
(-.57) (-.12) (-6.31)

40. 

Cephalotoxane

$2f_{CH} + 2f_{C} + 2f^{*}_{C} + 3f^{\phi}_{O} +$ (1)
(.355) (.13) (.40)(-.57)
$f^{\phi}_{OH}{}^{(2)} + 7f_{CH2} + 3f_{CH} + f_{C}$
(-1.63)(.66) (.43) (.20)
$+ f_{N} + f_{=} + f_{CH3} + 16f_{b} +$
(-2.16)(-.55)(.89)(-.09)
$+ f_{px1} + f_{px2}$ = 2.46 0.84
(.78) (.40)

(1) considering $-OCH_3$ as on aromatic.

(2) not considering α to vinyl as α to aromatic.

FIGURE 13

SUMMARY

It is rather satisfying to find that the partition coefficient due to the main hydrocarbon structure of a solute can be calculated from a constant for hydrogen of 0.23 and one for carbon of 0.20. Actually there are four 'Fundamental' constants in our method, the other two being a constant for each chain or ring bond (after the first) (Figure 14). With six 'ancillary' constants, two each for branching, non-conjugated unsaturation, and enhancement of fusion carbons in aromatic rings, we can extend the calculations to all common structure types. However, to these ten constants we must add a host of values for the polar functional groups which must be further separated as to aliphatic or aromatic attachment or fusion in a ring (Figure 15). And furthermore, to cope with many of the biologically interesting molecules, a great number of interaction terms must be known.

A few years ago calculating log Ps 'from scratch' appeared to be a hopeless task, unless 'ballpark' estimates would suffice. We still cannot recommend 'from scratch' values for use in regression analysis, but we are making progress towards that goal.

'FUNDAMENTAL' APPROACH TO FRAGMENT CALCULATIONS

A SUMMARY

FUNDAMENTAL CONSTANTS	
(1) f_H =	0.23
(2) f_{CH_3} =	0.89
(3) f_b =	-0.12
(4) $f_{\underline{b}}$ =	-0.09

ANCILLARY CONSTANTS

Branched Aliphatic

(1') f_{cbr} = -0.13

(2') f_{gbr} = -0.22

Aromatics

remove bond factor by ↓

Non-Conjugated Unsaturates

(3') $\Delta f_{=}$ = -0.10

(4') Δf = -0.50

$$\left(\text{Log P}_{\hexagon} = 6f_{CH2} + 5f_{b} - 6f_{H} = 2.13;\ f_{\underline{CH}} = 0.35_5\right)$$

$$6(.89-.12)+\ 5(-.09)-6(.23)$$

(5') $f\dot{\underline{C}}$ = 0.225

(6') $f^{*}_{\underline{C}}$ = 0.40

FIGURE 14

STRUCTURAL HYDROPHOBIC CONSTANTS

Chain Group	f	$f^{\phi(1)}$	$f^{\phi\phi(2)}$
-Br	0.20	1.09	
-Cl	0.06	0.94	
-F	-0.38	0.37	
-I	0.60	1.35	
$-N\langle$	-2.16	-1.17	-1.29*
$-NO_2$	-1.26	-0.02	
-O-	-1.82[a]	-0.57	0.53
-S-	-0.79	0.03	0.77
-NH-	-2.10	-1.03	-0.18
$-NH_2$	-1.54	-1.00[b]	
-OH	-1.63	-0.40[b]	
-CN	-1.28	-0.34	
$-\overset{O}{\overset{\|}{C}}-N\langle$	-3.20	-2.82	-2.09
$-\overset{O}{\overset{\|}{C}}-NH-$	-2.71	-1.81	-1.06
$-\overset{O}{\overset{\|}{C}}-$	-1.90	-0.32	-0.50*
$-\overset{O}{\overset{\|}{C}}-O-$	-1.49	-0.56	-0.09
$-\overset{O}{\overset{\|}{C}}-OH$	-1.09	-0.03	
(n-1)bonds	-0.12		
Chain branch	-0.13		
Group branch	-0.22		

Aliph. Ring

(n-1) bonds -.09

Ring Segment	f^{ϕ}
$-\overset{\bullet}{N}-$	-1.54
-N=	-1.12
$-N=^{+}$	-6.31
-O-	-0.07
-S-	0.38
-Se-	0.45
C	0.13
$\overset{\bullet}{C}$	0.22_5
$\overset{*}{C}$	0.40
CH	0.35_5
$\overset{O}{\overset{\|}{C}}$	-0.61
$\overset{O}{\overset{\|}{C}}-O-$	-0.82

(1) aromatic
(2) doubly aromatic
(a) does not apply to CH_3-O-CH_3.
(b) approx. .25 higher on α-naphthyl
* *not doubly enhanced as expected.*

$-OPO_3\langle$ -2.95 -2.88

$-OPSO_2\langle$ -1.03

$-PO_3H_2$ -1.36

FIGURE 15

STATISTICAL PROPERTIES OF ENVIRONMENTAL DATA: CONSIDERATIONS FOR BIOACCUMULATION AND TOXICITY STUDIES OF POLLUTANTS.

CHAPTER 10

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ABSTRACT

This paper discusses the characteristics and statistical properties of environmental data. The relationships among the problem(s), experiment(s) and data which affect the amount of information available from the experiment, and the sources and magnitudes of the errors, are described. Examples drawn from environmental data illustrate how such information can be used to study the distribution and bioaccumulation of toxicants. The multivariate techniques which are described can be extended to include molecular structural features.

The theory of Murkov-Polya Urn Models is applied to the development of a model of pollutant-induced stress on biological communities.

Apriori models for biotoxicity studies are available from drug design, where approaches[1] such as Hansch extrathermodynamic activity correlations, the Free-Wilson Additivity Model, molecular orbital studies, and cluster analysis have been used with varying degrees of success.

Hansch's approach, for example, uses simple mathematical equations which relate the biological activities of a series of closely related compounds to one or more physical parameters measured for these compounds. The parameters are independent and can be used singly or together, and linearly or quadratically, and many possible combinations can be considered. Using multiple regression techniques, biological activities are fitted to an equation of the form:[1,2]

$$(1) \quad \log A_i = k_1 (\log P_i)^2 + k_2 \log P + k_3$$

where A_i is the biological activity

P_i is an octanol water-partition coefficient

and the k's are regression coefficients.

In the Free-Wilson Additivity Model,[1] no assumptions are made concerning the physical parameters which may play a role in the biological activity "Instead," states Craig,[3] "a series of *de novo* substituent constants is obtained using only the experimentally obtained biological test data and the following basic assumption: every time a particular substituent group appears at the same place in the molecule, it is assumed that it will play a constant role towards determining the biological activity of the molecule." This assumption is checked by the statistical parameters obtained, by regression, from the solution of the equation:

$$(2)\quad A_i = \mu + \sum_{j=i}^{i} G_j X_j = \mu + A + B + C + \ldots\ldots Z$$

where A_i is the biological activity of the i^{th} compound

μ is the average biological activity, determined from the solution of the regression equation

$G_j X_j$ represents the activity contribution for the i^{th} group at the i^{th} position of the molecule.

X_j takes the values of 0 or 1 if the group is absent or present, respectively.

The statistical assumptions behind the use of linear regression models apply to these quations. The explicit assumptions are:

(1) Normally and randomly distributed observations of the dependent variable, y, for any given value of the independent variable, x.

(2) The independent variable, x, is measured without error.

(3) The expected value of the variable y (for a selected x) has a mean, μ and a constant variance σ^2. Equivalently, the errors (on y) have a normally distributed mean of 0 and a common variance of σ^2.

(4) For multiple regression, these assumptions take the form that both the independent and dependent variables together are distributed as multivariate normal with vector of means $\underline{\mu}$ and variance-covariance matrix, Σ.

When data do not meet these requirements, other regression techniques are available.[21, 22]

Data employed in such studies are obtained from laboratory investigations where factors such as dose, environment, diet, and the genetic heritage of the test animals, are carefully controlled. Even under these conditions, data can be of inconstant quality, or otherwise insufficient to permit unique solutions of the models. Since the careful laboratory studies needed to assess the biotoxicity of specific compounds of environmental interest are both long-term and expensive, inexpensive information which is available over the short term should be obtained and used in designing these studies. Such information exists in the living laboratories of natural waters.

Some of the statistical properties of environmental data are examined in this paper. We assume that the primary use of this data is the gathering of information about the effects of existing pollutants, rather than the design of new materials. The focus of this talk, therefore, is not biotoxicity, bioaccumulation, or structure, per se, but rather, the characteristics of the data produced in such studies.

With Nature as the laboratory it is more difficult to extract information from data, but conclusions drawn from such data may be better predictors of environmental variance or response. This dichotomy arises because the noise (random effects) inherent in random (environmental) data is always larger than in experiments designed to reduce random fluctuations. In general, less data is required in the latter situation, and the variability of such data as given by measures such as the variance, coefficient of variation, confidence interval, etc., is less than for completely random data. Thus, while environmental data is random, and requires more observations than laboratory studies to reach a given level of confidence, it measures the magnitude of the responses of organisms in real environments to real stresses.

Before proceeding, it is necessary to define some of the characteristics of environmental data. Thus,

1) Data is random, and the magnitudes of random and systematic errors are unknown. These errors fall into the categories of sample plus sampling, and analytical. Gross errors are ignored.

2) Temporal effects can be important.

3) Past history of the source is unknown. With organisms for example, factors such as the range of the organism, sex, age, species, while important, are not controlled in collecting random data.

4) Sampling technique affects the quality of data. Care should be taken to distinguish representative sampling which is based, for example, on careful hydrologic studies, from convenience sampling, which is based on what it is practical to collect. In the same way, random and arbitrary sampling must be distinguished. The former might require overlaying the collection area with a grid, and then randomly selecting specific sites. The latter approach might be to drop a net at locations selected because they were more convenient to get to, etc. As another example, consider an aquarium containing "N" fish. If the animals are arbitrarily numbered from 1 to "N" at the beginning of the experiment, then specimens can be selected by true random sampling techniques[4] as the experiment progresses.

5) Statistical distributions of data must be determined. Much environmental data has been assumed to follow the normal or lognormal distributions,[5] although in many instances this is not true.

6) Environmental data is multivariate, but most analyses, such as the Hansch and Free-Wilson models, are univariate.[1] Thus, more than one character is measured, e.g., age (weight), sex, species, source of sample (flesh, fat, organ), chemical parameters (PCB, DDT, etc.).

7) The most important consideration is for the investigator to properly define and to define proper questions. Thus, is it both possible and practical to obtain answers to the questions being asked? Are correct experiments being

designed to provide the information necessary to answer the questions? Have data analysis techniques been determined in advance, and are they adequate to answer the questions? The weakness of many (most) experiments is the failure to plan in advance the techniques for data analysis, insuring that the amount and quality of data is sufficient, and analyzing it by techniques appropriate to both the data and the problems (Figure 1).

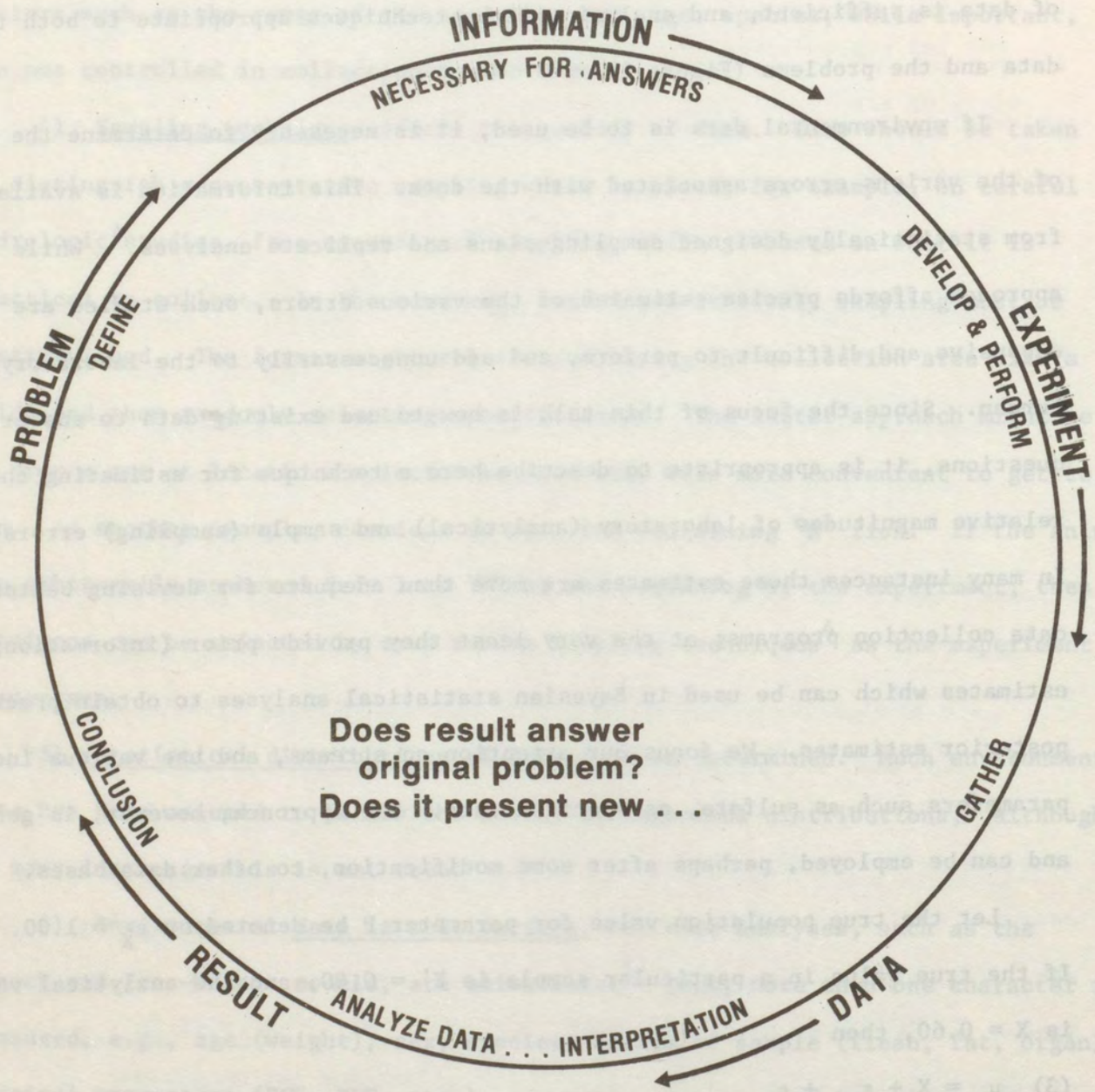
If environmental data is to be used, it is necessary to determine the magnitudes of the various errors associated with the data. This information is available from statistically designed sampling plans and replicate analyses.[4] While this approach affords precise estimates of the various errors, such studies are expensive and difficult to perform, and add unnecessarily to the laboratory burden. Since the focus of this talk is how to use existing data to answer new questions, it is appropriate to describe here a technique for estimating the relative magnitudes of laboratory (analytical) and sample (sampling) errors. In many instances these estimates are more than adequate for devising better data collection programs; at the very least they provide prior (information) estimates which can be used in Bayesian statistical analyses to obtain precise posterior estimates. We focus our attention on streams, and use various inorganic parameters such as sulfate, as specific data. The approach, however, is general, and can be employed, perhaps after some modification, to other data bases.

Let the true population value for parameter P be denoted by $\mu_X = 1.00$. If the true value in a particular sample is $X' = 0.80$, and the analytical value is $X = 0.60$, then

(3) $\mu_X = X + \varepsilon_{X'} + \varepsilon_X$

where $\varepsilon_{X'}$ is the sample error and ε_X is the analytical error. These errors represent all systematic and random errors. In this example

Figure 1: DATA FLOW



(4) $\varepsilon_{X'} = 1.00 - 0.80 = 0.20$

(5) $\varepsilon_{X} = 0.80 - 0.60 = 0.20$

and

(6) $\mu_{X} = 1.00 = 0.60 + 0.20 + 0.20$

The purpose of this exercise is to show that the various errors can be considered to be additive to a first approximation without losing generality. Without going into the mathematical details, if an estimate of ε_x is available from quality control data, such as the replication error of the method as applied to real samples of the type being evaluated,[6] an estimate of the total error is available as follows:

For the ith value of water quality for parameter p at the jth station, we write:

(7) $X_{ijp} = \mu_{ijp} + \varepsilon_{ijp}$ (ε_{ijp} is the error in the ith value obtained at the jth station for parameter p)

and

(8) $\varepsilon_{total} = |S_{ijp}|$

where $|S_{ijp}|$ is the value of the determinant of the variance-covariance matrix over all values of i,j,p.

Then,

(9) total error = sample error + analytical error

and

(10) sample error/analytical error = (total-analytical) error/analytical error

One important difference between laboratory and environmental data is that the latter may show marked seasonal and/or diurnal trends. Dissolved oxygen and temperature are two common examples,[7] but the bioaccumulation of organics might be expected to be seasonally related since the levels in the organism can vary with factors such as fat accumulation and diet. The seriousness of temporal variability must be evaluated against the specific questions being asked of the data. This is a good example of the necessity of planning the data analysis before samples are collected, since correcting for temporal variations

requires more and additional kinds of data, such as sampling time and flows, than analyses not requiring such corrections. One way to examine the magnitudes of such effects is presented later.

Numerical data can be subjected to statistical analysis. Statistical analyses either require the raw or transformed data to be normally distributed for parametric analysis, or demand some loss in confidence (greater variance) if no distributional assumptions are made.[8] Unfortunately, most experimentalists are not statisticians, and do not have a real understanding of the kind of information a statistician needs. Conversely, most statisticians are not sufficiently conversant with the experimenter's field to understand or recognize the pitfalls in data from a new experiment, and tend to fill the gaps in their knowledge with textbook cases they understand, without really knowing the validity of that particular model to this particular data.[9] For example, the experimenter really runs a paired experiment, but his description of the experiment to the statistician implies no pairing. This is a common example, and it can arise simply because their concepts[8] of "paired" are different. Another common example is the difference between the experimental and statistical concepts of "random" data, where the scientist frequently confuses "arbitrary" with "random" sampling.

Most data are assumed to follow either the normal or lognormal distributions. Simple tests of this assumption, applicable to small data sets, include plots on probability paper or tests based on the studentized range. It is our intention here to briefly review some reports which suggest that in many instances these assumptions are grossly in error. J. K. Ord, for example, in a study of "probabilistic models used in geology to describe concentrations of different elements in igneous rocks...(found) that the beta distribution is to be preferred (theoretically) to the more popular lognormal distribution."[10] He has also described applications of the negative binomial distribution to quadrat sampling, and has specifically investigated the validity of the Poisson

and generalized Poisson models to such sampling schemes.[11] In an unpublished study of asbestos fibers in Lake Michigan, we found that fiber counts by electron microscopy fit a negative binomial distribution. Kryukov has discussed the theoretical justification of Pearson Type III curves in hydrologic studies.[12] We have found that BOD, COD, and ammonia, among others, are also distributed in this fashion, rather than as normal or lognormal distributions. For small samples (n<200), however, the lognormal provides an approximate fit to BOD and COD data. Janardan[13] has discussed chance mechanisms which give rise to multivariate hypergeometric models, and discusses models applicable for haemocytometer counts, sampling for categorical data from a finite population, pollen analysis, among others. Both PCBs and total DDT in Lake Michigan fish (without regard to species) follow a Pearson Type I (Beta) distribution. For trout (all species), these same distributions obtain.[14] These latter findings are preliminary, and must be confirmed with additional data.

The binomial, Poisson and negative binomial distributions have been used extensively in drug-dose mortality studies. In the application of these distributions, it is usually assumed that a specific toxic effect has a constant probability which remains the same throughout a geographical area, time interval and type of species.

Talwalker[19] has recently questioned the validity of this assumption and has described a new model in toxicology based on Neyman's type A distribution.

In the concluding section of this paper we provide a model of biotoxicity which is applicable to natural communities

The points we want to emphasize here are:

(1) The distribution of the data must be determined.

(2) The distribution probably will not be normal or lognormal.

(3) Parametric correlations, such as those of Hansch, may suffer if they are

not robust to changes in the distribution.

Having focused on the attributes of environmental data, we now look briefly at some techniques of analysis, both univariate and multivariate.

(1) Pattern recognition techniques, under which we include cluster analysis, binary pattern classifiers, and information theory approaches such as distance classifiers, have been used with success in drug design. As stated by Kowalski,[15] "Pattern recognition...provides connections between raw, multivariant data and sought for information without making restrictive assumptions about the underlying statistics of the data....The only assumption made is that similarities and dissimilarities among objects are reflected in at least some of the measurements."

(2) Multivariate analysis of variance, MANOVA.

Whenever more than one measurement is made on a single object, the measurements cannot be considered <u>apriori</u> to be independent, and the covariance of the measurements cannot be assumed to be negligible. Thus, it becomes necessary to partition the total variance-covariance matrix into variance-covariance matrices due to the various factors of the experiment. With one dependent variable and n independent variables, the usual technique is multiple regression. When all the variables are dependent, regression techniques cannot be used. In these circumstances, the multivariate analog of the simple "t" and F tests are appropriate. These are available as Hoetling's T^2 and MANOVA tests.[20] As an example, consider an experiment where 2 species of fish (trout, salmon), are collected in two seasons (spring, fall). The fish are separated within each species by sex, and the heart and fat of each organism are analyzed for o,p'-DDD, o,p'-DDT, p,p'-DDD and p,p'-DDT. The MANOVA takes the form:

Statistical analysis of the multivariate factorial design of Table 1, requires the calculation of the sums, sums of squares, and sums of products, as shown in columns 3 to 10 of Table 2.

FIGURE 2: MANOVA LAYOUT

FACTORS:

	1	2
A. SEASON	Spring,	Fall
B. ORGAN	Heart,	Fat
C. SPECIES	Trout,	Salmon
D. SEX	Male,	Female

RESPONSES: $y^{(1)}$ $y^{(2)}$ $y^{(3)}$ $y^{(4)}$

OP - DDD OP - DDT PP - DDD PP - DDT

TABLE 1: Data Layout for Multi variate Factorial Experiment.

FACTORS				RESPONSES			
A	B	C	D	$y^{(1)}$	$y^{(2)}$	$y^{(3)}$	$y^{(4)}$
1	1	1	1	$y_1^{(1)}$	$y_1^{(2)}$	$y_1^{(3)}$	$y_1^{(4)}$
1	1	1	2	$y_2^{(1)}$	$y_2^{(2)}$	$y_2^{(3)}$	$y_2^{(4)}$
1	1	2	1	$y_3^{(1)}$			$y_3^{(4)}$
1	1	2	2	$y_4^{(1)}$			$y_4^{(4)}$
1	2	1	1				
1	2	1	2				
1	2	2	1				
1	2	2	2				
2	1	1	1				
2	1	1	2				
2	1	2	1				
2	1	2	2				
2	2	1	1				
2	2	1	2	$y_{14}^{(1)}$			$y_{14}^{(4)}$
2	2	2	1	$y_{15}^{(1)}$			$y_{15}^{(4)}$
2	2	2	2	$y_{16}^{(1)}$	$y_{16}^{(2)}$	$y_{16}^{(3)}$	$y_{16}^{(4)}$

Table 2: MANOVA TABLE FOR DATA IN TABLE 1

Source	df	$y^{(1)}$	$y^{(1)}y^{(2)}$	$y^{(1)}y^{(3)}$	$y^{(1)}y^{(4)}$	$y^{(2)}$	$y^{(2)}y^{(3)}$	$y^{(3)}y^{(4)}$	$y^{(3)}$	$y^{(3)}y^{(4)}$	$y^{(4)}$
A											
B											
C											
D											
AB											
AC											
AD											
BE											
BD											
CD											
ABC											
ABD											
ACD											
BCD											
ABCD											

(3) Other techniques which do not seem to have received much attention in toxicological investigations, and which may be valuable in general or specific applications, are response surface studies and Fourier transform analysis. The latter has been used at least once with environmental data, where it was desired to discern short and long term trends in climate from high background noise levels. We think that environmental data such as that considered in this paper or in some of the toxicological studies presented by others, should be examined by Fourier transform analysis, but have not as yet initiated such studies.

Response surface techniques might provide visual, as well as numerical, tools for toxicological studies. Using simplex optimization as an example, the vertices of the simplex might be molecular features which are changed in a regular way, while the biological activity is the response to be optimized. This technique is achieving some importance in developing optimized analytical techniques,[16] and it would seem that its usefulness in other applications may be limited only by imagination.

In drawing this talk to a close, we would like to focus briefly on a theoretical model which attempts to explain the statistical behavior of a population presented with a toxic pollutant.[18]

Consider two urns marked 1 and 2. Urn 1 contains 'a' white balls and urn 2 contains 'a' white balls and 'b' red balls. Three integers, 'n', 't' and 'c' are arbitrarily picked, e.g., by Nature. A fourth integer 'k' which determines the strategy is then selected (by the scientist) according to the following rules:

(1) At the outset, add 'kt' red balls to the contents of urn 1, and 'kt' white balls and '(n-k)t' red balls to urn 2. If the ball is red, no further picks are made.

(2) Pick one ball from urn 1. If the ball is white pick 'n' balls one at a time from urn 2.

(3) After each pick from urn 2, replace the ball in urn 2 along with 'c'

additional balls of the same color.

(4) Count the total number of white balls observed in the 'n' trials.

The number, k, of white balls observed will have a distribution called the quasi Markov-Polya Distribution, as given by the equation:

$$P(k) = \frac{a}{a+kt} \quad \frac{n!}{k!(n-k)!} \quad \frac{\prod_{j=0}^{k-1}(a+kt+jc) \prod_{j=0}^{n-k-1}(b+(n-k)t+jc)}{\prod_{j=0}^{n-1}(a+b+nt+jc)}$$

Now, let 'a' be the number in the first species; and 'b' the number in a second species which competes with the first. 'c' is the (common) immigration rate of the two species to a particular region in which species one and two already exist. 'n' is the increase in population, for both original species, due to reproduction,'t' is an environmental effect. Negative 't' corresponds to a factor which results in diminishing the population, such as a toxic material, while positive 't' is a factor favoring increase in the population, such as improved food supply. Under these conditions, the effects of changes in 't' on the population, as given by 'k', is determined by this probability model.

In conclusion, in evaluating the significance of data, we must evaluate the validity of experimental protocol, the reliability of the tests used to make the measurements, the quality of the answers obtained from the data analysis and their relation to the original questions, and the significance of the conclusions. Paraphrasing Jurs,[2] the establishment of structure-activity relationships does not necessarily indicate that the molecular properties and the toxic responses "are related by a cause and effect relationship to these parameters, but only that these parameters have a high degree of usefulness in the mathematical discrimination of these properties as they pertain to this data set."

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AN APPROACH TO THE STUDY OF MULTIPLE TOXICITY

CHAPTER 11

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Aquatic organisms inhabiting the receiving waters of man-made wastes are commonly exposed to several discrete toxicants simultaneously. Where such mixtures are concerned, the possibility of interplay between toxic constituents - either involving kinetic (i.e. uptake, accumulation, elimination) or dynamic (i.e. mode of action) mechanisms - may occur. The interplay may result in a mixture being more toxic than would be predicted on the basis of an appreciation of the potency of each of its constituents. Consequently, water quality standards when based solely on an assessment of tolerance to individual chemical contaminants do not necessarily safeguard aquatic life from mixtures.

A rationale which allows for the prediction of the toxicity of mixtures through the derivation and use of quantal (all or none) response curves of the toxic constituents is proposed. The validity of the approach is empirically tested and its usefulness is an aid in providing water quality criteria for mixtures is evaluated.

The rationale assumes three principle categories of toxic action between discrete chemical constituents of a mixture. The three types have been designated independent action, additive action and interaction (i.e. synergism and antagonism) respectively.

The independent action category is predictable on the assumption that the kinetic and dynamic mechanisms of each toxic constituent are unique and are not influenced by any other chemical component present in the mixture. The magnitudes of the toxic response to binary mixtures of dieldrin (HEOD) and potassium pentachlorophenate (KPCP) were predicted in accordance with the assumptions of this model. The contributions which HEOD and KPCP made to the common response induced by their mixture were computed from the discrete quantal response curves representing their pure solutions. The significance of this category of response is that when constituents are present in a mixture to levels which respectively are known to be below threshold (i.e. safe), then no toxic response to the mixture occurs.

The additive action type is predictable on the assumption that the dynamic mechanism is common to or similar for all toxic constituents. Individual toxic components may differ in their relative potencies or efficacies but act in an identical manner on the target tissue. Consequently the quantal response curves for each constituent as well as their corresponding mixture should be parallel. Parallel quantal response curves were demonstrated for discrete solutions of copper

and nickel and for their binary mixtures. The copper and nickel in the binary mixtures contributed to the total effect in proportion to their relative potency. A survey of the literature confirms the additive action of copper and nickel as well as other combinations of heavy metals. Toxicants which in a mixture demonstrate additive action are a serious threat to aquatic organisms because sub threshold levels (i.e. safe) of each constituent may add to produce an adverse effect.

The last type of toxic action of mixtures, designated interaction, implies that the kinetics and/or dynamics of a toxic constituent are altered in the presence of another toxicant. The relative potency of a toxicant may be either enhanced (synergism) or lessened (antagonism) in the mixture. An example of this category of response was found in our experiments. The magnitude of the response to a mixture in which the components are interacting is dependent on the relative proportions of the constituents rather than on their inherent potencies as depicted for the other two models. In a fixed proportion, the combined constituents act as a single toxicant resulting in a quantal response curve unique in slope compared to the curve of each of the constituents.

TOXICITY INDEX FOR PERMITS

CHAPTER 12

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ABSTRACT

Recently interest has been expressed in developing a rationale whereby the toxicity limits for organic compounds could be determined by structure for water pollution control purposes. The possibility of a relationship between percent composition and toxicity were explored since such a relationship would readily lend itself to the permitting situation. An intensive review led to the conclusion that structure is far more important in determining toxic effects than percent elemental composition, thus the hypothesis had to be rejected.

The fact that small differences in chemical structures can significantly influence the biologic effects of chemicals makes it most difficult to accurately forecast the toxicity of compounds to aquatic-organisms by extrapolation from exposure tests on alternate chemicals. It is proposed that a coefficient of relative potency can be derived based on the toxic units concept. This approach to the problem of experimental prediction of adverse effects by use of a reference substance for which toxicity to aquatic organisms is known for a particular structure is of possible use. Specific calculations based on tolerance level median data are presented for four phenols.

"Knowledge is of two kinds. We can know a subject ourselves, or we know where we can find information upon it."

Samuel Johnson, April 18, 1775, from Boswell's "Life of Johnson," published 1791.

The National Field Investigations Center is the technical service arm of the Office of Enforcement for the U.S. Environmental Protection Agency. As such, it is one of our responsibilities to assist the Office of Enforcement in the development of permits. Our Federal Law PL 92-500 requires that all effluent dischargers have a permit setting forth the nature of the discharge, such as BOD and pH.

In recent months considerable interest has been expressed at the National Field Investigations Center-Denver in developing a rationale to affix discharge limits for organic compounds to permits. We decided to explore the relationship between percent composition and toxicity since such a relationship would readily lend itself to permitting. Also, since our Chemistry Branch can easily analyze for specific constituents such as phosphorus, nitrogen, halogens or carbon, compliance monitoring for percent composition of these compounds would then be straightforward. We also could reasonably expect these analyses by the permittees in their self-reporting data.

The toxicity side of this relationship was the more hazy one. We know, for example, that in the series

$$H_3C\ Cl < H_2\ C\ Cl_2 < H\ C\ Cl_3 < C\ Cl_4$$

the toxicity increased with the amount of chlorine present, carbon

tetrachloride being the most toxic. We thought that there might be considerable toxicity data available to evaluate this concept if we surveyed the available literature. To keep such a survey sufficiently comprehensive but within the constraints of available time, we reviewed the following classes of nitrogen compounds.

Table 1. COMPOUNDS SELECTED FOR TOXICITY EVALUATION

Class	Member	
nitro	nitromethane	nitroethane
	nitropropane	nitropentane
	nitrobenzene	nitrotoluene
	glyceryl trinitrate	nitronaphthalene
amines	methylamine	ethylamine
	propylamine	aniline
	phenylene diamine	toluylamine
	naphthylamine	napthalenediame
nitriles	acetonitrile	propionitrile
	acrylonitrile	benzonitrile
	adiponitrile	
amides	acetamide	benzamide
	propionamide	butyramide
heterocycles	pyridine	pyrimidine
	pyrrole	imidazole
	pyrozole	quinoline
	carbazole	pyrrolidine

We developed this list with our Chemistry Branch as their initial work with total nitrogen analysis was most promising.

The review produced a moderate amount of toxicological information for the majority of these compounds, but for some there was very little. However, because of the diverse nature of the information gathered, it soon became apparent that a review of the basic elements of toxicology is necessary to synthesize the data into a meaningful format for permit applications.

At this point it is desirable to examine the proposition initially put forward that percent composition is related to the toxicity of the compound.

Figure 1

IDEALIZED EXAMPLE ILLUSTRATING POSSIBLE TOXICITY RELATIONSHIP

Compounds Nitro A, -B, -C, -D, and Dinitro E contain only $-NO_2$ as a contributing factor to their toxicity. The following tables illustrate the possible constant relationship between quantity of nitrogen present and the toxicity of the compound.

TABLE 1

Compound	Mol. Wt.	TLM_{96} (mg/kg)
Nitro A	156	22.3
Nitro B	198	28.3
Nitro C	246	35.1
Nitro D	293	41.8
Dinitro E	282	20.1

Table 1 doesn't seem to show any constant relationship in the toxicity of the various compounds. Yet, with a little number-juggling, a definite relationship appears.

TABLE 2

Compound	Fraction N (mg N/mg)	TLM_{96}* (mg N/kg)
A	0.0897	2.0
B	0.0707	2.0
C	0.0569	2.0
D	0.0478	2.0
E	0.0993	2.0

* $TLM_{96} = TLM_{96}$ x mg N/mg

Thus, in this idealized example, when the toxicity of a group of compounds is reexpressed in terms of the toxicity producing agent we find a constant relationship so that we need only to measure the amount of N present to determine the toxicity of a solution of any mixture of these compounds. While this perfect relationship is not likely with "real" compounds, a somewhat similar situation could prevail.

Unfortunately, the model's assumptions usually are not true in the overall scope of toxicity where structure is far more important in determining toxic effects than percent elemental composition. Nonetheless, we need to incorporate a toxicity or adverse effects rationale into the permitting process, although the task is difficult and the conclusions are more obscure than is legally desirable. Some answers are available and intelligent attempts at regulation are still possible.

The principles of toxicologic methodology are based on the premise that all effects of chemicals on living tissues are the result of a reaction with or interaction between any given chemical energy and some component of the biologic system. This initial reaction may not be evident. The result of this reaction is manifested as an effect on the function, and in many cases, the structure of the biologic system. The effect on function may not necessarily be accompanied by a detectable change in the structure of the biologic system. That is, it may only be a biochemical lesion. The effect may or may not be reversible if exposure to the chemical is discontinued.

Toxicological methodology is centered on the detection and evaluation of the chemical-induced changes in the function and structure and the significance of these effects on living cells. Since all effects of chemicals on living systems are not necessarily harmful effects, a principal objective of toxicology is to identify those chemicals capable of seriously harming living systems. As a science, toxicology has developed a methodology to detect chemical-induced alterations in function and structure of living systems; to investigate many of the factors that determine how chemicals gain access to biological cells; to establish the conditions under which various chemicals do or do not produce

biologic effects; and to define the mechanisms by which chemicals interact with the various components of living systems in order to directly or indirectly produce toxic effects.

As a result of the development of this methodology, certain general principles have become recognized. These principles apply to many and perhaps all toxicologic test procedures. They are as follows:[1/]

1. In order for a chemical agent to produce a biologic effect, it must come into immediate contact with the biological cells under consideration.
2. There will be some quantity of each chemical below which there will be no detectable effect on biologic systems, and there will be some greater amount of each chemical at which a significant effect will be present in essentially all biologic systems. Within this range are levels that will produce significant effects on some types of biological systems.
3. Cells having similar functions and similar metabolic pathways in various species generally will be similarly affected by a given chemical entity.
4. Last, and most significant to this report, small changes in the structure of a chemical agent may greatly influence the biological action of that agent.

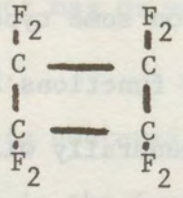
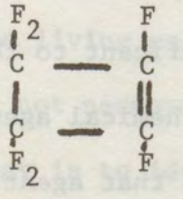
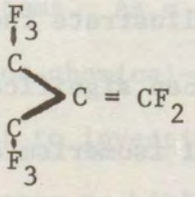
The following examples illustrate the principle that small differences in chemical structures can significantly influence the biologic effects of chemicals. Optical isomerism affects biologic action of the drug amphetamine (racemic-β-phenyl-isopropylamine). In mammals, this compound has several hormone effects, central nervous system stimulation,

and stimulation of receptors which are normally enervated by the sympathetic nervous system. The d-isomer is three to four times more potent than the l-isomer in its action on the central nervous system, whereas the l-isomer is about two times more potent in its action on the heart.

An example of the effect of valence on toxicity is shown by arsenic. The difference in the lethal toxicity of trivalent arsenic as compared to the pentavalent arsenic is not very great in mammals, but the difference is considerable in lower animals and plants. The trivalent arsenites are much more lethal for protozoa, bacteria and yeast than are the pentavalent arsenates.

A good example of the influence of structure and valence is given by the fluorocarbons in Table 2.

Table 2. FLUOROCARBON TOXICITY

Percent Fluorine	Compound Structure	Toxicity
76%		4 hr LC_{50} rat >800,000 ppm
66%		4 hr LC_{50} rat = 5 ppm
76%		4 hr LC_{50} rat <0.5 ppm

Thus, by a change of 10 percent composition in fluorine and replacing a single carbon bond with a double carbon bond but leaving the basic structure untouched, the toxicity expressed as a 4-hour LC_{50} for rat changes more than five orders of magnitude. On the other hand, with no change in percent composition of fluorine but a change in basic structure, the toxicity has changed by six orders of magnitude. It therefore will often be necessary to forecast toxicity based on structure.

Another illustrative example is the work of Yoshikawa who studied the aliphatic nitriles, one of the classes of compounds selected for investigation.[2/] He studied the acute toxicity of aliphatic nitriles and the mechanism of appearance of their toxic symptoms in mice. The compounds studied were: acetonitrile, propionitrile, butyrionitrile, capronitrile, and acrylonitrile, methacrylonitrile, lactonitrile, acetone cyanohydrin, and ethylene cyanohydrin. Of the alkyl nitriles, acetonitrile and propionitrile were most toxic. The toxicity decreased as the number of carbon atoms in the alkyl chain increased. Subacute toxic symptoms, e.g., convulsions, on the other hand, were intensified as the number of carbon atoms increased. The toxic effects of the alkylnitriles were not related to the release of cyanide from the parent molecule but rather due to the intact molecule itself while those of the acrylonitrile were due to cyanide release.

In a related study, Soeda and Yamamota studied the relation of structure to toxicity of the pyridylalkylamines.[3/] They obtained toxicity data by a topical application of the insecticides to houseflies. Tabulated results indicated that the primary amine compounds were almost non-insecticidal, whereas the secondary and tertiary ones

were insecticidal for the series of N-mono- and N, N-dialkyl 3-pyridylmethylamines. The insecticidal activity of the dialkyl derivative was similar to that of the mono-alkyl compound. The importance of the high basicity of the nitrogen was illustrated by the fact that the N-(3-pyridylmethyl)-morpholine which has a low basic nitrogen was very low in toxicity, while structurally similar but highly basic compounds such as N-(3-pyridylmethyl)-triperidine and N-(3-pyridylmethyl) pyrrolidine were highly insecticidal.

The percent composition of the isomers p-cresol and o-cresol is exactly the same; however, the TL_m 1-hour for bluegills is 90 mg/l for p-cresol, but only 65 mg/l for o-cresol. Likewise, the oral rat LD_{50} is 1800 mg/Kg for p-cresol, but only 1350 mg/Kg for o-cresol. If toxicity were related to percent composition, it would be identical for these two compounds.

One more phenolic example is the isomeric compounds o-nitrophenol and p-nitrophenol. A review of the toxicity data for these two compounds reveals that for oral dog LD_{50} or threshold to *Daphnia, Scenedesmus or Microregma*, both are considerably more toxic in the ortho form than in the para form.

In homologous nitromethane and nitroethane, the percent composition of nitrogen decreases from 22.9 to 18.6 percent from nitromethane to nitroethane, yet the oral rat LD_{50} increases from 900 mg/Kg to 1100 mg/Kg. Likewise, in the homologous series ethylamine, propylamine and butylamine the highest oral toxicity LD_{50} to rats is 570 mg/Kg for propylamine, with a lesser value for both ethylamine, 400 mg/Kg, and butylamine, 442 mg/Kg. So, as percent nitrogen decreased from 31 percent

to 19 percent, oral toxicity increased and then decreased.

Finally, in the case of the nitrogen-containing heterocycles pyrozole has a 41 percent nitrogen composition and an intraperitoneal LD_{50} to mice of 5.38 mg/Kg. Carbazole is composed of only 8 percent nitrogen, yet its intraperitoneal LD_{50} to mice is only two and a half times less than that for pyrozole. Likewise, quinoline containing 11 percent nitrogen has an oral LD_{50} to rats of 460 mg/Kg, while pyridine has an oral toxicity LD_{50} to rats of 1580 mg/1 and contains 18 percent nitrogen. It is apparent that there are many cases for fluorides and nitrogen compounds in which there is little or no correlation between percent composition and toxicity.

In spite of the limitations in predicting toxicity there are some excellent examples of the results which may be achieved in the future when data are more carefully prepared and synthesized. Kowalski and Bender devised a computerized method of screening potential anticancer drugs for their therapeutic activity.[4/] The technique, which has proven more than 90 percent accurate in predicting antitumor activity in a class of drugs previously known to be of value, is based upon chemical pattern recognition.

While 90 percent success is spectacular, only two structurally similar groups about which a great deal of information is already known were investigated, and the predictions required the use of a large-scale computer program. To apply such a program across-the-board to the permit program is far beyond the technical capabilities of the National Field Investigations Center-Denver.

We seem to have arrived at an impasse. On one hand toxicology has

produced much data on the adverse effects of chemicals on living organisms, but on the other hand, has not produced a unifying principle which would enable us to use these data for our primary purposes. We have to introduce a vehicle to enable us to make rational judgments on permit applications. The rationale presented here borrows certain elements from a number of toxicology theories and rearranges them so that we can make a utilitarian calculation.

Horvath and Frantik approached the problem of experimental prediction of adverse effects by trying to develop a rationale for a reference substance.[5/] This reference procedure involves three steps. First, the concentrations (or doses) producing the same effect on an organism are determined from dose-response curves for both the new and the reference substances. In optimum cases the concentration of the new substance provokes the same effect as the concentration or dose of the reference substance. Second, the coefficient of relative potency is derived. This is the ratio of equally effective concentrations of the reference versus the unknown substance. Third, the product of the reciprocal of the coefficient and of the concentration for the reference substance represents a test specific estimate of the threshold value for the new substance under study.

The validity of this procedure, especially the third step, is based on several assumptions and conditions pertaining to the test and reference substances. It is assumed that the ratio of experimentally determined effects of both substances correlates with the ratio of their hazard to an organism. This assumption may not always be justifiable, because the conditions of experimental exposure and the mechanism of biological

action of the two substances which are compared may differ in actual exposures in the ecosystem.

In applying this reference substance methodology to the testing of a new compound with an unknown mode of action, the question arises as to which type of biological effect should be used in experimental studies. I chose death so that TL_m's could be used. Selection of a reference substance fitted to the special type of effect is of key importance. Application of the reference principle is difficult because of the diversity of toxic symptoms. Horvath and Frantik felt that the principle use of the reference substance would be to allow different laboratories using common reference substances to compare and complement their data and also to help estimate the effectiveness of different methods.

The number of manipulations done in Figure 1 are similar to the third step of Horvath and Frantik's procedure. In both cases an attempt was made to produce a product of a reciprocal of a coefficient of relative potency (adverse effect). Horvath and Frantik tried unsuccessfully to use the level of concentration (or dose). We were not able to use tolerance level median (TL_m) (a more meaningful index of adverse effects for our purposes) because we tried to relate relative potency to percent structural composition as noted earlier.

Relating the coefficient of relative potency based on tolerance level median structural (functional) grouping such as all nitro, amines, nitriles, amides, or heterocycles may provide the solution. For example, assume that for two amine compounds, ethylamine and propylamine, the acute oral toxicity to rats is known. For ethylamine the TL_m 96-hour

to creek chub is also known. The coefficient of relative potency (a ratio of the acute oral toxicity to rats for ethylamine to its TL_m 96-hour value for creek chub) is the number by which the acute oral toxicity to rats for propylamine is multiplied to provide the TL_m 96-hour value for creek chub. An example is given later in Figure 3. This calculation accesses more toxicity data since the amount of TL_m information available is small in comparison to the body of toxicological information. It must be remembered that this is only an extrapolation in an attempt to synthesize information from the mass of data by combining like groups.

The reason for attempting to convert toxicological information to TL_m values is to take advantage of the concept of toxicity units proposed by Sprague[6/] and refined by Esvelt, Kaufman and Selleck.[7/] This concept expresses toxicity as the percent of total waste found in a solution rather than the concentration of a specific substance. The reciprocal of the tolerance level median gives a numerical index of toxicity concentration increasing with increasing toxicity:

$$T_c = \frac{100}{TL_m \text{ 96-hour percent}} \qquad \text{Equation (1)}$$

where T_c is the toxicity concentration in toxic units (TU). By definition, a toxicity concentration in effluent of 1 toxic unit (TU) corresponds to 50 percent survival.

Pearson, *et al.*[8/] proposed a toxicity emission rate (TER) or relative toxicity which may be expressed as

$$TER = T_c \times Q \qquad \text{Equation (2)}$$

in TU-mgd or TU-cu m/day where Q is the total effluent flow.

The toxicity concentration may be expressed as the sum of the individual toxicity units. The toxicity concentraion in the receiving water, $(T_c)_r$, from a number of sources may be conveniently calculated as

$$(T_c)_r = (T_{c_1} Q_1 + T_{c_2} Q_2 + \ldots)/Q_t \qquad \text{Equation (3)}$$

where Q_t is the total flow, including the total waste discharge where the tolerance level median is determined by a bioassay run on the total waste discharge, not individual components within the waste.

The "Water Quality Control Plan for the State of California for Ocean Waters" includes the toxicity unit concept. The applicable guideline approved by EPA on August 18, 1972, reads as follows:

> "This parameter (TU) shall be used to measure the acceptability of waters for supporting a healthy marine biota until improved methods are developed to evaluate biological response."[97]

The State Water Resources Control Board of California has set the final toxicity concentration at not greater than 0.05 TU, while effluent concentration shall not exceed 1.5 TU more than 50 percent of the time or more than 2.0 TU more than 10 percent of the time.

The use of toxicity units should have intrinsic appeal in application to the permits program since it reduces the whole concept of toxicology to a single number. What it loses in sensitivity by arbitrarily defining a toxicity unit is compensated for by its ability to be incorporated into a permit. There may be more acceptance of the incorporation of a toxicity unit limitation than there would be of requirements based on the analysis of diverse organic compounds or the need to perform bioassays constantly.

It should be possible to extend the TU concept into a chemical measurement requirement by first determining the structural or functional types of compounds being discharged and then combining the TL_m's (for example, nitriles and amides) using a relative potency coefficient concept if necessary with flow data to calculate the toxicity emission rate. The treatment level imposed upon the discharger determines the number of toxic units allowed, which is the value attached to the permit.

Since the toxicity unit discharge level is based on the selection of structurally and functionally similar groups, one can calculate the amount of nitrogen which could be discharged to reach a particular number of toxicity units. This assumes that all the nitrogen in the discharge is accounted for in the nitrile and amines and that toxicity is realted solely to the presence of the nitrogen in these functional groups, which is not always true. Because we no longer have the restriction that the nitrogen must be the same relative toxicity from compound to compound within the group, a condition of the permit could be that the permittee must monitor the level of nitrogen in his discharge, a relatively simple procedure.

Self-reporting data would be used to indicate when a reassessment of toxic unit discharge was required. For example, if the permittee changed product from one amide to another, possibly changing the nitrogen discharged, this might mean that the toxicity unit level of discharge has changed. This could be calculated by determining TL_m values for the new amide products discharged.

In arriving at these conclusions we have assumed that toxicity

units may be summed together in a signal discharge. While no one appears to have addressed this problem as yet, other investigators have found that a variety of toxic effects are empirically additive.

Brown[10] estimated the acute toxicity to rainbow trout of mixtures of the common industrial pollutants: ammonia, phenol, zinc, copper, cadmium, lead, nickel and hydrogen cyanide. The proportional toxicity of each pollutant was obtained by dividing its concentration in the water by the TL_m 48-hour

$$\frac{\text{concentration of pollutant A in solution}}{TL_m \text{ 48-hour for pollutant A}} = \text{Proportion of A to the total lethal concentration}$$

Equation (4)

and similarly for pollutants B, C, D and so on. Values obtained for all the pollutants are then summed to give the proportion of the TL_m 48-hour of the pollutants in the mixture. If the sum is less than 1.0 it was considered that less than half the rainbow trout in a test batch would die in 48 hours, but if the value is greater than 1.0 that more than half would die in 48 hours.

Basically the method described by Brown assumes that all pollutants contribute similarly to the overall toxicity of a mixture although it is illogical to expect pollutants of different toxicological properties and different concentration-response curves to sum in this manner. Nevertheless, the method was found to work empirically.

Brown suggested that the pollutants can be regarded as agents producing stress, each of which produces a degree of shock with resulting nonspecific effects. The summation of overall stress may be possible.

Several other investigators have found this method applicable to

mixtures of two pollutants in aerated water under constant control conditions.[11, 12, 13, 14] The method has also been found to be reasonably accurate for mixtures of three[15] and four[16] pollutants at constant concentrations. Studies on more complex mixtures such as sewage effluents[17] and fluctuating concentrations of pollutants in rivers[18] indicated, however, that toxicity tended to be underestimated by summation of the proportions and that 50 percent of the fish die when the sum averaged about 0.7.

Smyth *et al.*[19] determined the oral rat LD_{50} value for 50 percent by volume mixtures of all possible pairs among 27 commercial organic chemicals of large volume use such as aniline, butylether, ethylacetate, nitrobenzene, propylene, glycol, and tetrachloroethylene. The soundest hypothesis for the joint action of untested pairs was that of additive toxic action.

While the studies cited were not specifically directed to the additive value of toxicity units there would seem to be no real objection to applying these results to the addition of toxicity units. With this approach in mind, toxicity units for compounds proposed for the initial study will be calculated based on the available literature. Since the State of California has already set a final toxicity concentration of not more than 0.05 toxicity units and this has been approved by EPA, we will start with this value. However, the California value was based on final dilution. Except in those cases that are water quality limiting, we propose that any effluent concentration shall not exceed 0.05 TU.

When dealing with aquatic discharges it seems reasonable and prudent to use TL_m values for aquatic organisms. The TL_m 96-hour should be the

standard TL_m for defining toxicity units. This is not a new recommendation. Water Quality Criteria in 1968 recommended that an arbitrary application factor of 1/100 of the TL_m 96-hour be used as the criterion of permissible level in the absence of other toxicity data.[20/] This value of 1/100 of the TL_m 96-hour is comparable with 0.05 toxic units. However, since the TL_m 96-hour is not always available some fraction of a 48-hour or 24-hour TL_m could be equated to the 96-hour TL_m.

For some of the chemicals proposed for investigation numerous TL_m values have been determined for a variety of vertebrate and invertebrate aquatic organisms. Because of interspecific differences in reaction to chemicals discussed previously, these TL_m values may vary significantly. Averaging of TL_m's in some cases will result in too many toxicity units being permitted so that the most sensitive species are not fully protected. Also, given the number of assumptions and unknowns already discussed, only a rough estimate can be obtained.

The data available on the proposed nitrogen-containing compounds is given in Figures 2-6. The scarcity of data is a fact which will be addressed later. In figure 3 the extension of known TL_m data for ethylamine to propylamine was mentioned earlier. For both of the substances an oral rat LD_{50} has been reported by different investigators. In addition, a creek chub TL_m 48-hour has been reported for ethylamine. The value or a fraction thereof could be considered to be a TL_m 96-hour as previously discussed. The ratio of the creek chub TL_m to the oral rat LD_{50} for ethylamine, gives a coefficient of relative potency of 0.1. This coefficient times the oral rat LD_{50} of propylamine gives the creek

Figure 2.

TOXICITY UNITS CALCULATIONS FOR NITRO COMPOUNDS

Name	Formula	Percent Composition	TLm's and Coefficients of Relative Potency	Other Toxic Properties
Nitromethane	CH_2NO_2	C - 19.67 H - 4.95 N - 22.9 O - 52.42		Oral, human LD_{50}, 500-5000 mg/kg[21] Oral, guinea pig ~LD_{50}, 1000 ppm[22] Oral, rat LD_{50}, 900 mg/kg[23] Oral, mouse LD_{50},950 mg/kg[23]
Nitroethane	$C_2H_5NO_2$	C - 32.00 H - 6.71 N - 18.66 O - 42.63		Oral, rabbit ~LD_{50}, 500 mg/kg[24] Oral, rat LD_{50}, 1100 mg/kg[23] Oral, mouse LD_{50}, 860 mg/kg[23]
Nitropropane	$C_3H_7NO_2$	C - 40.44 H - 7.92 N - 15.72 O - 35.92		Oral, rabbit LD_{50}, 250 mg/kg[23] Oral, human LD_{50}, 500-5000 mg/kg[25]
Nitrobenzene	$C_6H_5NO_2$	C - 58.53 H - 4.09 N - 11.38 O - 25.99		Oral, rabbit LD_{50},700 mg/kg[22] Oral, human LD_{50}, 5-50 mg/kg[26] Sewage organisms TC_{50}, BOD, 630 mg/l[27]
nitrotoluene	$C_7H_7NO_2$	C - 61.31 H - 5.15 N - 10.21 O - 23.33		Causes liver injury, methemoglabinemea[22]
Nitronaphthalene	$C_{10}H_7NO_2$	C - 69.36 H - 4.07 N - 8.09 O - 18.48		
Nitroglycerine	$C_3H_5N_3O_9$	C - 11.54 H - 3.87 N - 53.84 O - 30.75		Oral, rat LD_{50}, 80 mg/kg[27] Oral, rat LD_{50}, 100 mg/kg[27] Intramuscular, rat LD_{50}, 250 mg/kg[28] Intravenous, rabbit LD_{50}, 45 mg/kg[28] Oral, human LD_{50}, <5 mg/kg[29]

Figure 3.

TOXICITY UNITS CALCULATIONS FOR AMINE COMPOUNDS

Name	Formula	Percent Composition	TLm's and Coefficients of Relative Potency	Other Toxic Properties
Methylamine	CH_3NH_2	C - 38.67 H - 16.23 N - 45.10	Creek chub, TLm 24 hr., 20 mg/l.[35] $\frac{\text{(ethylamine) creek chub, TLm 48 hr.}}{\text{(ethylamine) Scenedesmus, threshold 96 hr.}} =$ $\frac{\text{(methylamine) creek chub, TLm 48 hrs.}}{\text{(methylamine) Scenedesmus, threshold 96 hr.}}$ $\frac{40}{10} = \frac{\text{(methylamine) creek chub, TLm 48 hr.}}{4}$ (methylamine) creek chub, TLm 48 hr. = 16 mg/l	Rainbow trout, 141 mg/l LD_{100}, 20 minutes[30] Scenedesmus, 4 mg/l, 96 hr., threshold[31] Microregma, 50 mg/l, 96 hr., threshold[31] Daphnia, 480 mg/l, 48 hr. threshold[31] Algae, 100 mg/l, LD_{100}, 120 hr.[32] Subcutaneous, rat, LD_{50}, 2500 mg/kg[22]
Ethylamine	$C_2H_5NH_2$	C - 53.28 H - 15.65 N - 31.07	Creek chub, TLm 48 hr., 40 mg/l.[35] $\frac{40}{10} = \frac{\text{Scenedesmus}}{} = 4$ $\frac{40}{400} = \text{oral, rat} = .1$	Sunfish, 400 mg/l LD_{100}, 1 hr.[33] Scenedesmus, 10 mg/l 96 hr. threshold[31] Microregma, 40 mg/l, 96 hr. threshold[31] Oral, rat, LD_{50}, 400 mg/kg[22]
Propylamine	$C_3H_7NH_2$	C - 60.95 H - 15.35 N - 23.70	$\frac{\text{(ethylamine) creek chub, TLm 48 hr.}}{\text{(ethylamine) oral, rat, } LD_{50}} =$ $\frac{\text{(propylamine) creek chub, TLm 48 hr.}}{\text{(propylamine) oral, rat, } LD_{50}}$ $\frac{40}{400} = \frac{\text{(propylamine) creek chub, TLm 48 hr.}}{570}$ (propylamine) creek chub, TLm 48 hr. = 57 mg/l	Oral, rat, LD_{50}, 570 mg/kg[34]

Figure 3. (Continued)

Name	Formula	Percent Composition	TLm's and Coefficients of Relative Potency	Other Toxic Properties
Butylamine	$C_4H_9NH_2$	C - 65.69 H - 15.16 N - 19.15	$\frac{\text{(ethylamine) creek chub, TLm 48 hr.}}{\text{(ethylamine) oral, rat, } LD_{50}} =$ $\frac{\text{(butylamine) creek chub, TLm 48 hr.}}{\text{(butylamine) oral, rat, } LD_{50}}$ $\frac{40}{400} = \frac{\text{(butylamine) creek chub, TLm 48 hr.}}{500}$ (butylamine) creek chub, TLm 48 hr. = 50 mg/l	Oral, rat, LD_{50}, 500 mg/kg[36] Inhalation, rat, LD_{50}, 4000 ppm[37]
Aniline	$C_6H_5N_2$	C - 77.38 H - 7.58 N - 15.04	Sunfish, TLm 1 hr., 1020 mg/l	Oral, rat, LD_{50}, 442 mg/kg[23] Inhalation, rat, LD_{50}, 250 ppm[23] Immobilized Daphnia magna, 279 ppm[38] Algae, Microcystis aeraginosa, LD_{100}, 120 hr., 50 ppm[32] Oral, mouse, LD_{50}, 1075 mg/kg[39] Fathead minnow, TLm 96 hr, 200 ppm[40] Goldfish, TLm 96 hr., 1000 ppm[40] Trout, TLm 96 hr., 1000 ppm[40] Daphnia magna, TLm 48 hr., 0.4 mg/l[31] Scenedesmus, TLm 96 hr., 10 mg/l[31] Oral, human, LD_{50}, 50-500 mg/Kg[41] Oral, dog, LD_{50}, 500 mg/Kg[22]

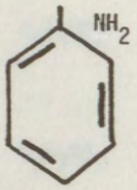


Figure 3. (Continued)

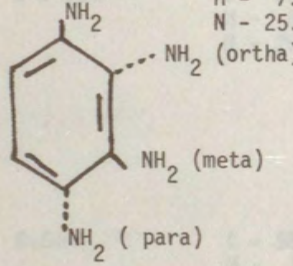
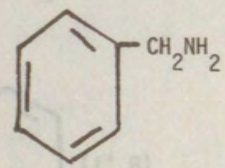
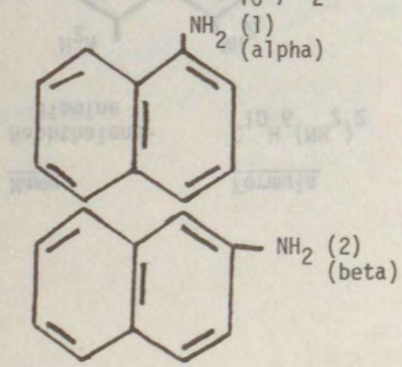
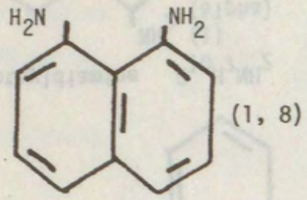
Name	Formula	Percent Composition	TLm's and Coefficients of Relative Potency	Other Toxic Properties
Phenylene diamine	$C_6H_4(NH_2)_2$ 	C - 66.64 H - 7.46 N - 25.91		Oral, rabbit, LD_{50}, 300 mg/Kg, (meta)[42] Subcutaneous, rat, LD_{50}, 600 mg/Kg (ortha)[43] Oral, rat, LD_{50}, 1700 mg/Kg (para)[44] Daphnia magna, LD_{100}, 48 hr., 5.74 ppm[45] Oral, human, 50-500 mg/Kg[46]
Toluylamine	$C_7H_7NH_2$ 	C - 78.46 H - 8.43 N - 13.07		Daphnia magna, TLm 48 hr. 60 ppm[47] Scenedesmus, TLm 96 hr., 6 ppm[47]
Naphthyldiamine	$C_{10}H_7NH_2$ 	C - 83.88 H - 6.34 N - 9.78		Subcutaneous, dog, LD_{50}, 400 mg/Kg (alpha)[48] Intraperitoneal, mouse, LD_{50}, 200 mg/Kg (beta)[22] Algae, toxic, 7 days, 2 ppm[49]

Figure 3. (Continued)

Name	Formula	Percent Composition	TLm's and Coefficients of Relative Potency	Other Toxic Properties
Naphthalene-diamine	$C_{10}H_6(NH_2)_2$	C - 75.92 H - 6.37 N - 17.71		



(1, 8)

Figure 4.

TOXICITY UNITS CALCULATIONS FOR NITRILE COMPOUNDS

Name	Formula	Percent Composition	TLm's and Coefficients of Relative Potency	Other Toxic Properties
Acrylonitrile	C=C-C≡N	C - 67.90 H - 5.70 N - 26.40	Fathead minnows, TLm 96 hr., 18.1 mg/l[47] Bluegills, TLm 96 hr., 11.8 mg/l[47] Guppy, TLm 96 hr., 33.5 mg/l[47] Pin perch, TLm 24 hr., 24.5 mg/l[50] Brine shrimp, Crangon, TLm 24 hr., 10 mg/l[31]	Inhalation, rat, LC_{50}, 500 ppm[54] Oral, rat, LD_{50}, 93 mg/Kg[33] Pin perch, threshold, 20 mg/l[51] Mixed fish, threshold, 38-68 mg/l[52] Mixed fish, threshold, 20-25 mg/l[53] Mixed fish, LD_{100}, 24 hr., 100 mg/l[53] Pin perch, LD_{100}, 24 hr., 100 mg/l[49] Oral, human, LD_{50}, 50-500 mg/Kg[54]
Acetonitrile	C-C≡N	C - 58.51 H - 7.37 N - 34.12	Fathead minnows, TLm 96 hr., 1000 mg/l[47] Bluegill sunfish, TLm 96 hr., 1850 mg/l[47] Guppy, TLm 96 hr., 1650 mg/l[47]	Oral, rat, LD_{50}, 3800 mg/Kg[23] Inhalation, rat, LC_{50}, 8000 ppm[31] Subcutaneous, rabbit, LD_1, 130 mg/Kg[23]
Propionitrile	C_2-C≡N	C - 65.42 H - 9.15 N - 25.43	$\frac{\text{(acetonitrile) fathead minnow TLm 96 hr.}}{\text{(acetonitrile) inhalation, rat } LC_{50}} =$ $\frac{\text{(propionitrile) fathead minnow, TLm 96 hr.}}{\text{(propionitrile) inhalation, rat, } LC_{50}}$ $\frac{1000}{8000} = \frac{\text{(propionitrile) fathead minnow, TLm 96 hr.}}{500}$ (propionitrile) fathead minnow, TLm 96 hr. = 62 mg/l $\frac{\text{(acetonitrile) bluegill sunfish TLm 96 hr.}}{\text{(acetonitrile) inhalation, rat, } LC_{50}} =$ $\frac{\text{(propionitrile) bluegill sunfish, TLm 96 hr.}}{\text{(propionitrile) inhalation, oral, } LC_{50}}$ $\frac{1850}{8000} = \frac{\text{(propionitrile) bluegill sunfish, TLm 96 hr.}}{500}$ (propionitrile) bluegill sunfish TLm 96 hr. = 115 mg/l $\frac{\text{(acetonitrile) guppy, TLm 96 hr.}}{\text{(acetonitrile) inhalation, rat } LC_{50}} =$ $\frac{\text{(propionitrile) guppy, TLm 96 hr.}}{\text{(propionitrile) inhalation, rat, } LC_{50}}$	Oral, rat, LD_{50}, 39 mg/Kg[55] Inhalation, rat, LC_{50}, 500 ppm[55]

Figure 4. (Continued)

Name	Formula	Percent Composition	TLm's and Coefficients of Relative Potency	Other Toxic Properties
Benzonitrile	C_6-C≡N	C - 81.53 H - 4.89 N - 13.59	Fathead minnow, TLm 96 hr., 135 mg/l [47] Bluegill sunfish, TLm 96 hr., 78 mg/l [47] Guppy, TLm 96 hr., 400 mg/l [47]	Subcutaneous, mouse, LD_{50}, 180 mg/Kg [23] Mixed fish, no effect, 24 hr., 5 mg/l [56]
Adiponitrile	N≡C$(CH_2)_4$ C≡N	C - 66.69 H - 7.40 N - 25.91	Fathead minnow, TLm 96 hr., 1250 mg/l [47] Bluegill sunfish, TLm 96 hr., 720 mg/l [47] Guppy, TLm 96 hr., 775 mg/l [47]	Intraperitoneal, mouse, LD_{50}, 40 mg/Kg [57]

Figure 5.

TOXICITY CALCULATIONS FOR AMIDES COMPOUNDS

Name	Formula	Percent Composition	TLm's and Coefficients of Relative Potency	Other Toxic Properties
Acetamide	CH_3CONH_2	C - 59.07 H - 8.53 N - 23.72 O - 27.09	Mosquito-fish, Gambusia, TL_m 96 hr. 13,300 mg/l[58]	Oral, rat, LD_{50} = 30 g/kg[22] Oral, rat, carcinogenic, 130 mg/kg, threshold[59]
Benzamide	$C_6H_5CONH_2$	C - 69.41 H - 5.82 N - 11.56 O - 13.21		
Propionamide	$C_2H_5CONH_2$	C - 49.30 H - 9.65 N - 19.17 O - 21.89		Inhalation, rat LD_{100}, 8000 ppm[22]
Butyramide	$C_3H_7CONH_2$	C - 55.14 H - 10.41 N - 16.08 O - 18.36		Intraperitoneal, mouse, 800 mg/kg, threshold[60]

Figure 6.

TOXICITY CALCULATIONS FOR HETEROCYCLES

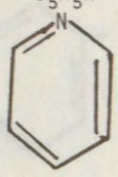
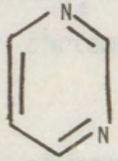
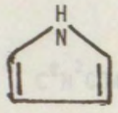
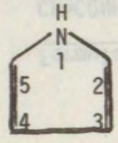
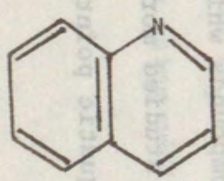
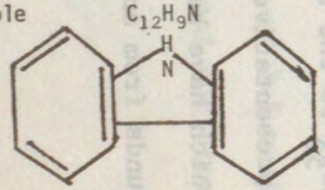
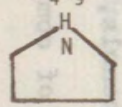
Name	Formula	Percent Composition	TLm's and Coefficients of Relative Potency	Other Toxic Properties
Pyridine	C_5H_5N 	C - 75.92 H - 6.37 N - 17.71	Mosquito fish, 96 hr. TLm, 130 mg/l[58] Goldfish, 24 hr TLm, 1830 mg/l[63] Daphnia, 48 hr. TLm, 944 mg/l[62]	Inhalation, rats, LD_{100}, 4000 ppm[22] Oral, rat, LD_{50}, 1580 mg/kg[53] Daphnia, 40 mg/l, threshold[31] Alburnus, 100 mg/l, threshold[64] Bleak, 160 mg/l, threshold[65] Bream, 180 mg/l, threshold[65] Carp, 200 mg/l, threshold[65] Escherichia coli, 200 mg/l, threshold[31]
Pyrimidine	$C_4H_4N_2$ 	C - 59.98 H - 5.03 N - 34.98		
Pyrrole	C_4H_5N 	C - 71.60 H - 7.51 N - 20.89		Subcutaneous, mice, LD_{50}, 60.5 g/kg[22]
Imidazole	$C_3H_4N_2$ 	C - 52.92 H - 5.92 N - 41.15		Oral, mouse, LD_{50} 1880 mg/kg[66]

Figure 6. (con't.)

TOXICITY CALCULATIONS FOR HETEROCYCLES

Name	Formula	Percent Composition	TLm's and Coefficients of Relative Potency	Other Toxic Properties
Pyrozole	$C_3H_4N_2$ 	C - 52.92 H - 5.92 N - 41.15		Intraperitoneal, mouse, LD_{50}, 538 mg/kg [67]
Quinoline	C_9H_7N 	C - 83.69 H - 5.46 N - 10.85		Oral, rat, LD_{50}, 460 mg/kg [67] Bluegill sunfish, LD_{100}, 4 hrs. 5.0 mg/l [56] Fish, LD_{100}, 7.5 mg/l [68] Yearling trout, LD_{100}, 10 mg/l, 1 hrs. [69] Perch, LD_{100}, 50 mg/l [70] Microregma, 50 mg/l, threshold [71] Daphnia, 52 mg/l, threshold [33] Scenedesmus, 140 mg/l, threshold [31] E. coli, 600 mg/l, threshold [31] Ciliates, LD_{100}, 700 mg/l [71]
Carbazole	$C_{12}H_9N$ 	C - 86.19 H - 5.43 N - 8.38		Oral, rat, LD_{50}, 5 g/kg [22] Intraperitoneal, mouse, LD_{50}, 200 mg/kg [72]
Pyrrolidine	C_4H_9N 	C - 67.55 H - 12.76 N - 19.70		

chub TL_m 48-hour for propylamine. This calculation is subject to the assumptions and limitations already pointed out.

Comparing calculated results with empirical results gives us some measure of the reliability of this method. In the case of the creek chub (Figure 3), a TL_m 24-hour has been determined for methylamine. Secondly, a 96-hour threshold toxicity level has been determined for *Scenedesmus* for both methylamine and ethylamine. In this case, the coefficient of relative potency for ethylamine has a value of 4 which when multiplied by the 4 mg/1 96-hour threshold value for *Scenedesmus* to methylamine, gives a TL_m 48-hour value of 16 mg/1. Since the creek chub TL_m 24-hour value to methylamine was 20 mg/1, one would expect it to be somewhat less for the TL_m 48-hour and 16 mg/1 is reasonable.

Again referring to Figure 3, the coefficient of relative potency of 0.1 for ethylamine using the TL_m 48-hour for creek chub and the oral LD_{50} to rats was used to determine a 50 mg/1 TL_m 48-hour for creek chub to butylamine.

Because in most cases little or no TL_m data is available for the nitrogen compounds selected, it is necessary to turn to other chemical groups to validate this rationale. This is difficult because of the scarcity of TL_m data available. We must select a sub-set which has TL_m values for the same time period and the same species of aquatic organism. This sub-set was still further limited in that adequate comparative data such as oral LD_{50} to the same mammal was not available for many of these compounds. Representative compounds which met these requirements were the phenols, which have been studied more consistently than any other group of compounds from an aquatic point of view, except pesticides.

Figure 7 lists the TL_m values and the calculations for some 14 phenolic compounds. In each case TL_m values have been empirically determined and these are compared with calculated TL_m values using the coefficient of relative potency. Phenol itself is taken as the base compound to which a variety of additional atoms or functional groups may be added. If the coefficient of relative potency is to be of real value, it should be able to overlap functional groups as well as to provide values throughout a homologous group.

Toxicity values and TL_m values are given for several fish; *Daphnia*, a crustacean; *Scenedesmus*, an alga; *Microregma*, a protozoan; and *E. coli*, a bacterium for the compound p-aminophenol. A TL_m 13-hour of 5 mg/l has been determined for bluegills. While no TL_m 13-hour was determined for bluegills to phenols, a 48-hour TL_m was determined and this is used as a comparative value. As seen in the TL_m coefficient of relative potency column for p-aminophenol, the ratio of the phenol bluegill TL_m 48-hour value to the phenol value for *Daphnia* threshold is compared to the ratio of the p-aminophenol bluegill TL_m 48-hour to the p-aminophenol *Daphnia* threshold value. The calculated bluegill TL_m 48-hour is 0.7 mg/l. Since the measured TL_m 13-hour for bluegills to p-aminophenol is 5 mg/l, we expect a TL_m 48-hour of 1 or 2 mg/l. The calculated TL_m value of 0.7 mg/l is only slightly less.

Using threshold values which have been determined for *Scenedesmus* for both phenol and p-aminophenol, the calculation under the p-aminophenol listing is a calculated bluegill TL_m 48-hour of 2.8 mg/l which is essentially the value expected. By using additional toxicity data, threshold

Figure 7.

TOXICITY CALCULATIONS FOR PHENOLS

Name	Formula	Percent Composition	TLm's and Coefficients of Relative Potency	Other Toxic Properties
Phenol	C_6H_5OH 	C - 76.57 H - 6.43 O - 17.00	Bluegill, TLm 1 hr., 70 mg/l[73] Bluegill, TLm 96, 11.5 mg/l[74] Bluegill, TLm 48 hr., 19 mg/l[75] Perch, TLm 1 hr., 9 mg/l[76] Mosquito fish, TLm 96 hr., 57 mg/l[58] Fathead minnow, TLm 48 hr., 40 mg/l[68] Trout, TLm 48 hr., 7.5 mg/l[77] Goldfish, TLm 96, 46 mg/l[78] Daphnia, TLm 24 hr., 6 mg/l[62] Daphnia, TLm 48 hr., 21 mg/l[62]	Oral, rat, LD_{50}, 530 mg/Kg[22] Daphnia, 16 mg/l, threshold[31] Scenedesmus, 40 mg/l, threshold[31] Microregma, 30 mg/l threshold[31] Oral, man, LD_{50}, 14 mg/Kg[79] E. coli, 1000 mg/l, threshold[31]
p-aminophenol	C_6H_7NO 	C - 66.03 H - 6.47 N - 12.84 O - 14.66	Bluegill, TLm 13 hrs., 5 mg/l[56] $\frac{\text{(phenol) bluegill, TLm 48 hr.}}{\text{(phenol) Daphnia, threshold}} =$ $\frac{\text{(p-aminophenol) bluegill, TLm 48 hr.}}{\text{(p-aminophenol) Daphnia threshold}}$ $\frac{19}{16} = \frac{\text{bluegill TLm 48 hr. (p-aminophenol)}}{0.6}$ (p-aminophenol) bluegill, TLm 48 hr. = 0.7 mg/l $\frac{\text{(phenol) bluegill, TLm 48 hr.}}{\text{(phenol) Scenedesmus, threshold}} =$ $\frac{\text{(p-aminophenol) bluegill, TLm 48 hr.}}{\text{(p-aminophenol) Scenedesmus, threshold}}$ $\frac{19}{40} = \frac{\text{(p-aminophenol) bluegill, TLm 48 hr.}}{6}$ (p-aminophenol) bluegill, TLm 48 hr. = 2.8 mg/l	Unreported, mouse, LD_{50}, 420 mg/Kg[80] Daphnia, 0.6 mg/l, threshold[31] Scenedesmus, 6 mg/l threshold[31] Daphnia, TLm 48 hr., 2 mg/l[45]

Figure 7. (Continued)

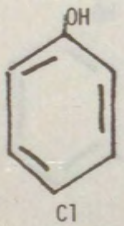
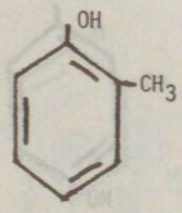
Name	Formula	Percent Composition	TLm's and Coefficients of Relative Potency	Other Toxic Properties
p-chlorophenol	C_6H_5ClO 	C - 56.05 H - 3.92 Cl - 27.58 O - 12.44	Bluegill, TLm 96 hr., 8.1 mg/l[81] $\frac{\text{(phenol) bluegill, TLm 96 hr.}}{\text{(phenol) oral, rat } LD_{50}} =$ $\frac{\text{(p-chlorophenol) bluegill, TLm 96 hr.}}{\text{(p-chlorophenol) oral, rat, } LD_{50}}$ $\frac{11.5}{530} = \frac{\text{(p-chlorophenol) bluegill, TLm 96 hr.}}{670}$ (p-chlorophenol) bluegill, TLm 96 hr. = 14.6 mg/l	Oral, rat, LD_{50}, 670 mg/Kg[22]
o-cresol	C_7H_8O 	C - 77.75 H - 7.46 O - 14.80	Bluegill, TLm 1 hr., 65 mg/l[81] Perch, TLm 1 hr., 20 mg/l[81] Bluegill, TLm 96 hr., 24 mg/l[78] $\frac{\text{(phenol) bluegill, TLm 1 hr.}}{\text{(phenol) oral, rat, } LD_{50}} =$ $\frac{\text{(o-cresol) bluegill, TLm 1 hr.}}{\text{(o-cresol) rat, } LD_{50}}$ $\frac{70}{530} = \frac{\text{(o-cresol) bluegill, TLm 1 hr.}}{1350}$ (o-cresol) bluegill, TLm 1 hr. = 176 mg/l $\frac{\text{(phenol) perch, TLm 1 hr.}}{\text{(phenol) oral, rat, } LD_{50}} =$ $\frac{\text{(o-cresol) perch, TLm 1 hr.}}{\text{(o-cresol) rat, } LD_{50}}$ $\frac{9}{530} = \frac{\text{(o-cresol) perch, TLm 1 hr.}}{1350}$ (o-cresol) perch, TLm 1 hr. = 23 mg/l $\frac{\text{(phenol) bluegill TLm 96 hr.}}{\text{(phenol) oral, rat, } LD_{50}} =$ $\frac{\text{(o-cresol) bluegill, TLm 96 hr.}}{\text{(o-cresol) oral, rat, } LD_{50}}$	Oral, rat LD_{50}, 1350 mg/Kg[28]

Figure 7. (Continued)

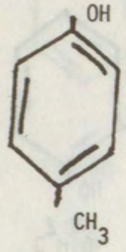
Name	Formula	Percent Composition	TLm's and Coefficients of Relative Potency	Other Toxic Properties
o-cresol (continued)			$\frac{11.5}{530} = \frac{\text{(o-cresol) bluegill, TLm 96 hr.}}{1350}$ (o-cresol) bluegill, TLm 96 hr. = 29.4 mg/l	
p-cresol	C_7H_8O 	C - 77.75 H - 7.46 O - 14.80	Bluegill, TLm 1 hr., 90 mg/l[81] Perch, TLm 1 hr., 20 mg/l[81] $\frac{\text{(phenol) bluegill, TLm 1 hr.}}{\text{(phenol) oral, rat, } LD_{50}} =$ $\frac{\text{(p-cresol) bluegill TLm 1 hr.}}{\text{(p-cresol) oral, rat, } LD_{50}}$ $\frac{70}{530} = \frac{\text{(p-cresol) bluegill TLm 1 hr.}}{1800}$ (p-cresol) bluegill, TLm 1 hr. = 228 mg/l $\frac{\text{(phenol) perch, TLm 1 hr.}}{\text{(phenol) oral, rat, } LD_{50}} =$ $\frac{\text{(p-cresol) perch TLm 1 hr.}}{\text{(p-cresol) oral, rat, } LD_{50}}$ $\frac{9}{530} = \frac{\text{(p-cresol) perch TLm 1 hr.}}{1800}$ (p-cresol) perch, TLm 1 hr. = 30 mg/l	Oral, rat, LD_{50}, 1800 mg/Kg[28]

Figure 7. (Continued)

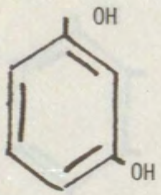
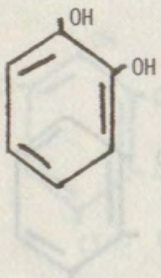
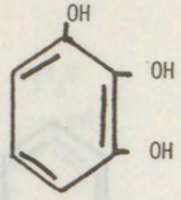
Name	Formula	Percent Composition	TLm's and Coefficients of Relative Potency	Other Toxic Properties
resorcinol	$C_6H_6O_2$ 	C - 65.44 H - 5.49 O - 29.06	Daphnia, TLm 48 hr., 55 mg/l[39] $\frac{\text{(phenol) Daphnia, TLm 48 hr.}}{\text{(phenol) oral, rat } LD_{50}} =$ $\frac{\text{(resorcinol) Daphnia TLm 48 hr.}}{\text{(resorcinol) oral, rat } LD_{50}}$ $\frac{21}{530} = \frac{\text{(resorcinol) Daphnia TLm 48 hr.}}{980}$ (resorcinol) Daphnia TLm 48 hr. = 39 mg/l	Oral, rat, LD_{50}, 980 mg/Kg[88]
pyrocatechol	$C_6H_6O_2$ 	C - 65.44 H - 5.49 O - 29.06	Perch, TLm 1 hr., 20 mg/l[87] $\frac{\text{(phenol) perch, TLm 1 hr.}}{\text{(phenol) oral, rat, } LD_{50}} =$ $\frac{\text{(pyrocatechol) perch, TLm 1 hr.}}{\text{(pyrocatechol) oral, rat } LD_{50}}$ $\frac{9}{530} = \frac{\text{(pyrocatechol) perch, TLm 1 hr.}}{3890}$ (phenol) perch, TLm 1 hr. - 66 mg/l $\frac{\text{(phenol) perch, TLm 1 hr.}}{\text{(phenol) Microregma, threshold}} =$ $\frac{\text{(pyrocatechol) perch, TLm 1 hr.}}{\text{(pyrocatechol) Microregma, threshold}}$ $\frac{9}{30} = \frac{\text{(pyrocatechol) perch TLm 1 hr.}}{6}$ (pyrocatechol) perch, TLm 1 hr. = 1.8 mg/l	Oral, rat, LD_{50}, 3890 mg/Kg[22] Microregma, 6 mg/l, threshold[31]

Figure 7. (Continued)

Name	Formula	Percent Composition
pyrogallol	$C_6H_6O_3$	C - 57.14 H - 4.80 O - 38.06



TLm's and Coefficient of Relative Toxicity

Goldfish, TLm 48 hr., 18 mg/l[83]

$$\frac{\text{(phenol) goldfish, TLm 96 hr.}}{\text{(phenol) Daphnia, threshold}} = \frac{\text{(pyrogallol) goldfish TLm 96 hr.}}{\text{(pyrogallol) Daphnia, threshold}}$$

$$\frac{46}{16} = \frac{\text{(pyrogallol) goldfish, TLm 96 hr.}}{18}$$

(pyrogallol) goldfish, TLm 96 hr. = 52 mg/l

$$\frac{\text{(phenol) goldfish, TLm 96 hr.}}{\text{(phenol) Scenedesmus, threshold}} = \frac{\text{(pyrogallol) goldfish, TLm 96 hr.}}{\text{(pyrogallol) Scenedesmus, threshold}}$$

$$\frac{46}{40} = \frac{\text{(pyrogallol) goldfish, TLm 96 hr.}}{8}$$

(pyrogallol) goldfish, TLm 96 hr. = 9.2 mg/l

Other Toxic Properties

Subcutaneous, rabbit, LD_{50}, 650 mg/Kg[83]
Daphnia, 18 mg/l, threshold[31]
Scenedesmus, 8 mg/l, threshold[31]
Microregma, 50 mg/l, threshold[31]

Figure 7 (Continued)

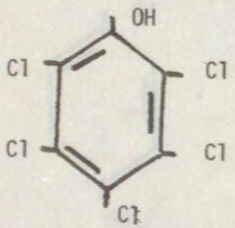
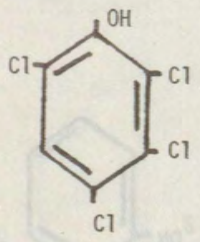
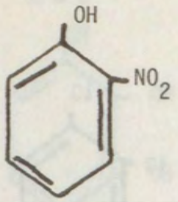
Name	Formula	Percent Composition	TLm's and Coefficients of Relative Potency	Other Toxic Properties
pentachloro-phenol	C_6HCl_5O 	C - 27.05 H - 0.38 Cl - 66.56 O - 6.01	Bluegill, TLm 3 hr., 5 mg/l [56] Crassostrea virginica egg, TLm 48 hr., 0.25 mg/l [85] $\frac{\text{(phenol) bluegill, TLm 1 hr.}}{\text{(phenol) oral, rat, } LD_{50}} =$ $\frac{\text{(pentachlorophenol) bluegill, TLm 1 hr.}}{\text{(pentachlorophenol) oral, rat, } LD_{50}}$ $\frac{70}{530} = \frac{\text{(pentachlorophenol) bluegill, TLm 1 hr.}}{78}$ (pentachlorophenol) bluegill, TLm 1 hr. = 10 mg/l	Oral, rat, LD_{50}, 78 mg/Kg [83]
2,3,4,6-tetra-chlorophenol	$C_6H_2Cl_4O$ 	C - 31.10 H - 0.86 Cl - 60.90 O - 7.24	Trout, TLm 4 hrs., 5 mg/l [56] $\frac{\text{(phenol) trout, TLm 48 hr.}}{\text{(phenol) oral, rat } LD_{50}} =$ $\frac{\text{(2,3,4,6-tetrachlorophenol) trout, TLm 48 hr.}}{\text{(2,3,4,6-tetrachlorophenol) oral, rat, } LD_{50}}$ $\frac{7.5}{530} = \frac{\text{(2,3,4,6-tetrachlorophenol) trout, TLm 48 hr.}}{140}$ (2,3,4,6-tetrachlorophenol) trout, TLm 48 hr. = 2.0 mg/l	Oral, rat, LD_{50}, 140 mg/Kg [86]

Figure 7. (Continued)

Name	Formula	Percent Composition
o-nitrophenol	$C_6H_5NO_3$	C - 51.80 H - 3.62 N - 10.07 O - 34.50



TLm's and Coefficients of Relative Potency

Bluegill, TLm 48 hr., 52 mg/l[90]

$$\frac{\text{(phenol) bluegill, TLm 48 hr.}}{\text{(phenol) Daphnia, threshold}} = \frac{\text{(o-nitrophenol) bluegill, TLm 48 hr.}}{\text{(o-nitrophenol) Daphnia, threshold}}$$

$$\frac{19}{16} = \frac{\text{(o-nitrophenol) bluegill, TLm 48 hr.}}{60}$$

(o-nitrophenol) bluegill TLm 48 hr. = 71 mg/l

$$\frac{\text{(phenol) bluegill, TLm 48 hr.}}{\text{(phenol) Microregma, threshold}} = \frac{\text{(o-nitrophenol) bluegill, TLm 48 hr.}}{\text{(o-nitrophenol) Microregma, threshold}}$$

$$\frac{19}{30} = \frac{\text{(o-nitrophenol) bluegill, TLm 48 hr.}}{40}$$

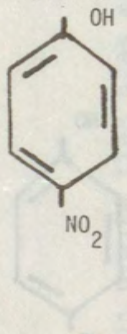
(o-nitrophenol) bluegill, TLm 48 hr. = 25 mg/l

Other Toxic Properties

Intravenous, dog, LD_{50}, 100 mg/Kg[22]
Daphnia, 60 mg/l, threshold[31]
Scenedesmus, 36 mg/l, threshold[31]
Microregma, 40 mg/l, threshold[31]

Figure 7. (Continued)

Name	Formula	Percent Composition
p-nitrophenol	$C_6H_5NO_3$	C - 51.80 H - 3.62 N - 10.07 O - 34.50



TLm's and Coefficients of Relative Potency

Bluegill, TLm 48 hr., 46 mg/l[89]

$$\frac{\text{(phenol) bluegill, TLm 48 hr.}}{\text{(phenol) Scenedesmus, threshold}} = \frac{\text{(p-nitrophenol) bluegill, TLm 48 hrs.}}{\text{(p-nitrophenol) Scenedesmus, threshold}}$$

$$\frac{19}{40} = \frac{\text{(p-nitrophenol) bluegill, TLm 48 hr.}}{72}$$

(p-nitrophenol) bluegill, TLm 48 hr. = 34 mg/l

$$\frac{\text{(phenol) bluegill, TLm 48 hr.}}{\text{(phenol) Microregma, threshold}} = \frac{\text{(p-nitrophenol) bluegill, TLm 48 hr.}}{\text{(p-nitrophenol) Microregma, threshold}}$$

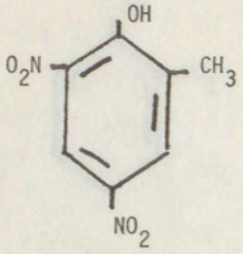
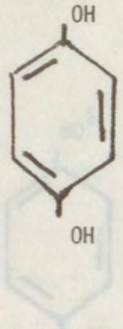
$$\frac{19}{30} = \frac{\text{(p-nitrophenol) bluegill, TLm 48 hr.}}{20}$$

(p-nitrophenol) bluegill, TLm 48 hr. = 13 mg/l

Other Toxic Properties

Intravenous, dog, LD_{50}, 83 mg/Kg[22]
Scenedesmus, 72 mg/l, threshold[31]
Microregma, 20 mg/l, threshold[31]
Daphnia, 14 mg/l, threshold[31]

Figure 7. (Continued)

Name	Formula	Percent Composition	TLm's and Coefficients of Relative Potency	Other Toxic Properties
4,6-dinitro-o-cresol	$C_7H_6N_2O_5$ 	C - 42.43 H - 3.05 N - 14.14 O - 40.38	Bluegills, TLm 13 hr., 5 mg/l[56] Naiads, TLm 96 hr., 0.00032 mg/l[82] $\frac{\text{(phenol) bluegill, TLm 48 hr.}}{\text{(phenol) oral, rat, } LD_{50}} =$ $\frac{\text{(4,6-dinitro-o-cresol) bluegill, TLm 48 hr.}}{\text{(4,6-dinitro-o-cresol) oral, rat, } LD_{50}}$ $\frac{19}{530} = \frac{\text{(4,6-dinitro-o-cresol) bluegill, TLm 48 hr.}}{30}$ (4,6-dinitro-o-cresol) bluegill TLm 48 hr. = 1.1 mg/l	Oral, rat, LD_{50}, 30 mg/Kg[84]
hydroquinone	$C_6H_4(OH)_2$ 	C - 65.44 H - 5.49 O - 29.06	Goldfish, TLm 48 hr., 0.287 mg/l[83] $\frac{\text{(phenol) goldfish, TLm 96 hr.}}{\text{(phenol) Daphnia, threshold}} =$ $\frac{\text{(hydroquinone) goldfish, TLm 96 hr.}}{\text{(hydroquinone) Daphnia, threshold}}$ $\frac{46}{16} = \frac{\text{(hydroquinone) goldfish, TLm 96 hr.}}{0.6}$ (hydroquinone) goldfish, TLm 96 hr. = 1.7 mg/l $\frac{\text{(phenol) goldfish, TLm 96 hr.}}{\text{(phenol) E. coli, threshold}} =$ $\frac{\text{(hydroquinone) goldfish, TLm 96 hr.}}{\text{(hydroquinone) E. coli, threshold}}$ $\frac{46}{1000} = \frac{\text{(hydroquinone) goldfish, TLm 96 hr.}}{50}$ (hydroquinone) goldfish, TLm 96 hr. = 2.3 mg/l	Daphnia, 0.6 mg/l, threshold[31] E. coli, 50 mg/l threshold[31]

values which were determined for a crustacean and an alga, we can calculate a reasonable TL_m for bluegills to p-aminophenol.

For p-chlorophenol a bluegill TL_m 96-hour of 8.1 mg/l has been experimentally determined. Using oral toxicity LD_{50} values to rats as the connecting toxicity link, the calculated bluegill TL_m 96-hour is 14.6 mg/l. The determination of this value is given in the TL_m's and coefficient of relative potency column for p-chlorophenol. This value is close to the experimentally determined value and, again, somewhat on its low side. The calculated value gives reasonable protection to the fishery and is valid for the calculation of toxicity units.

For the compound o-cresol three TL_m values were found in the literature, two for bluegills and one for perch. The calculations using rat LD_{50} to connect toxicity data are given. The calculated bluegill TL_m 1-hour is 176 mg/l versus the 65 mg/l reported from the literature. The correspondence between these values is good considering the variation expected in a 1-hour bioassay. The calculated TL_m 1-hour for perch is 23 mg/l versus that reported of 20 mg/l. The bluegill TL_m 96-hour was calculated to be 29 mg/l versus the measured value of 24 mg/l. In all three cases the calculated values again are slightly less than the experimental values.

For the compound p-cresol, 1-hour TL_m values were reported for bluegills and perch. The calculated value for bluegills was 228 mg/l, while the measured value was 90 mg/l. For perch the calculated value was 30 mg/l versus 20 mg/l determined in the laboratory. Both calculated values were well within the acceptable range, and slightly on the low side.

In the case of 4,6-dinotro-o-cresol, the calculated value was 1.1 mg/l for 48 hours while the given value was 5 mg/l for 13 hours; again, a reasonable correspondence. The system works quite well for the addition of functional groups such as amines, halogens, alkanes and nitros.

This system is also applicable to hydroxol groups, as shown by the calculations for hydroquinone. The TL_m values calculated using either *Daphnia* or *E. coli* were acceptable although on the low side, again demonstrating use of peripheral information of a diverse nature to calculate TL_m values as a predecessor to calculating toxicity units.

The compound pentachlorophenol has a reported TL_m 3-hour to bluegills of 5 mg/l. The corresponding calculation was made using the TL_m 1-hour bluegill value for phenol to give a value of 10 mg/l. While this value is double the reported toxicity and, therefore, on the high side, it is for a 1-hour TL_m and it is reasonable to expect it to be half that value for a 3-hour TL_m. There is considerable variation in TL_m values in these rapid, acute bioassay tests.

The calculated TL_m 48-hour for trout for the compound 2,3,4,6-tetrachlorophenol is 2 mg/l. This is high since the given TL_m 4-hour is 5 mg/l.

In the case of resorcinal a calculated TL_m 48-hour for *Daphnia* was 39 mg/l, while a measured value was 56 mg/l, indicating that the system is not restricted just to determining TL_m values for fish.

Pyrocatechol represents the first case given in which the calculated values span the determined value for perch, which was a TL_m 1-hour of 20 mg/l. Based on oral rat LD_{50} toxicity, the calculated value

is 66 mg/1. Although this value is more than three times that empirically determined, it is probably not unreasonable. However, the value calculated using *Microregma*, a protozoan, is less than 10 percent of the determined value and is unreasonably low. This divergence in values shows that the system is not perfect and that the more routes followed to calculate a TL_m value, the better the value will be.

In the next example, o-nitrophenol, both *Scenedesmus* and *Microregma* give reasonable values, although both are again on the low side. One can not single out any particular type of toxicity test as a consistent producer of erratic calculations. Another example of values having a range too high and too low is o-nitrophenol. The stated TL_m 48-hour for bluegills to o-nitrophenol. The calculated value using *Daphnia* threshold values is 71 mg/1, while the calculated value using *Microregma* threshold values is only 25 mg/1. However, neither of these values is particularly unreasonable.

Finally pyrogallol provides yet another example of the same sort. The stated TL_m 48-hour for goldfish to pyrogallol is 18 mg/1. The calculated value using *Daphnia* is 52 mg/1, while that using *Scenedesmus* is only 9 mg/1.

While none of the calculated values deviated unrealistically, undoubtedly some such examples can be found. In the case of litigation a TL_m value must be measured to give a realistic check against the calculated values. In many cases insufficient data exist on a compound to make any calculation, so we must always be able to measure TL_m values.

An example follows of the calculation for a plant which discharges acetonitrile, propionitrile and o-nitrophenol. These are all compounds which are detectable by nitrogen analysis. Referring to Figure 4, there are TL_m 96-hour acetonitrile values for fathead minnows, bluegill sunfish and guppies. Similar values for these three species of fish were calculated for propionitrile. For this calculation these TL_m values have been averaged together to provide a baseline TL_m for fish. We recognized that this may underprotect the most sensitive species and overprotect least sensitive species. If there is rationale to start with a TL_m value for the most sensitive species present in the calculation of toxicity units, then we can do that. The averaged TL_m 96-hour for fish to acetonitrile is 1500 mg/l, while that for propionitrile is 93 mg/l. From Figure 7 the only TL_m value available in the literature for o-nitrophenol is that of a TL_m 48-hour to bluegills of 52 mg/l. For computational purposes, this is translated to a 96-hour TL_m. With a standardized 96-hour TL_m test the concentration which is lethal to 50 percent of the exposed animals in a 96-hour period has a toxicity concentration of 1 toxic unit (TU). Thus, the averaged TL_m 96-hour of 1500 mg/l for acetonitrile is a toxicity concentraiton of 1 TU. Likewise, the TL_m 96-hour of 93 mg/l for propionitrile is a toxicity concentration of 1 TU and that of 52 mg/l for o-nitrophenol is also 1 TU.

The proposed standard that the final toxicity concentration shall not be greater than 0.05 TU means in effect that the sum of the toxic concentrations discharged for acetonitrile, propionitrile and o-nitrophenol is also 1 TU in the effluent.

Assume that a company is discharging a mixture of acetonitrile,[1/] propionitrile,[2/] and o-nitrophenol[3/] through a single outfall at the rate of 1,000,000 gallons per day. With other conditions and assumptions remaining the same we would have:

$$(T_c)_r = \frac{(T_{c_1}Q_1 + T_{c_2}Q_2 + T_{c_3}Q_3)}{Q_1 + Q_2 + Q_3} = 0.05 \text{ TU}$$

Equation (5)

$$(T_{c_1}Q_1 + T_{c_2}Q_2 + T_{c_3}Q_3) = 0.05 \text{ TU}$$

$$T_{c_1}Q_1 + T_{c_2}Q_2 + T_{c_3}Q_3 = 0.05 \text{ TU}$$

If either the permittee or National Field Investigations Center-Denver suspends a cage containing 100 fish or some other aquatic organism in the effluent stream from the plant and at the end of 96 hours 50 fish had died, this would be the TL_m 96-hour value and equivalent to 1 TU. This would be in violation of the permit which requires 0.05 TU maximum. If less than 1 fish died (essentially equal to control conditions) this would be 0.05 TU. It would not matter what concentrations of these chemicals occurred in the effluent so long as less than 1 fish died within a 96-hour period. This would assure that the toxicity concentration in the effluent would not exceed 0.05 TU.

At this point the measured nitrogen value for the chemicals or self-reporting data on this value would become the limit. As long as there was no significant change in the nitrogen value, we have reasonable assurance that an effluent toxicity concentration of 0.05 TU is not being exceeded. If nitrogen values change with process modifications, it may signal an increase in toxicity. It would then be necessary to

redo the bioassay or recalculate the TL_m 96-hour values. The proposed use of a TL_m 96-hour has been strengthened by our proposal that any element or compound can be considered as a candidate hazardous substance if it is lethal to one-half of a test population of aquatic animals in 96 hours or less at a concentration of 500 mg/l or less.[91/]

If the permit is water quality limited, calculations would be based on the total water available for dilution, Q_t. Assume that the plant discharges 1,000,000 gallons per day of waste containing only acetonitrile and water through one outfall to the river having a flow of 10,000,000 gallons per day. The toxicity concentration (T_c) is by definition one toxicity unit for a concentration of 1500 mg/l of acetonitrile. Also assume that the concentration in the receiving water $(T_c)_r$ must not exceed 0.001 toxicity units. In this case,

$$(T_c)_r = \frac{(T_c Q)}{Q_t} = 0.001 \text{ TU}$$

$$\frac{T_c \times 1}{(10 + 1)} = 0.001 \text{ TU} \qquad \text{Equation (6)}$$

$$T_c = 11 \times 0.001 \text{ TU}$$

$$T_c = 0.011 \text{ TU}$$

In order to meet the minimal expected dilution conditions, the toxicity concentration in the effluent for acetonitrile must not exceed 0.011 toxic units. In reality, there would be few places where the number of dischargers is so great and the volume of water available so small as to create this condition. These few permits could be handled better by other means.

How often do we need to run a bioassay? Reviewing the literature indicates that the answer is often, but less frequently than if we did

not calculate related TL_m 96-hour values. The literature is biased towards pharmaceutical and clinical applications, basic metabolic investigations, aspects of mutagenesis, terratogenesis, and carcinogenesis. Toxicity units are based upon the most definite biological measurement available -- death. The body of toxicity literature is vast, yet that which applies to determining mortality is small. As an example, the TOXLINE data base for nitrobenzene contains no less than 76 references on the adverse effects of this compound. Typical titles read as follows:

"Nitrobenzene Reduction and Reductive Cleavage of Azobenzenes in Two Species of *Arachnida*"

"Comparative Diagnostic Value of Various Pathological Derivatives of Hemoglobin in Conditions of Acute and Subacute Poisoning by Aniline, Nitrobenze and Their Chloride Derivatives"

"Radiosensitization of Mammalian Cells by p-Nitroacetophenone. III. Effectiveness of Nitrobenze Analogues"

"Adrenal Cortex Function in Chronic Nitrobenzene Poisoning of Guinea Pigs and the Effect of Hydrocortisone on the Course of Poisoning"

"Antifungal Activity of Substituted Nitrobenzenes and Anilines"

"Hematological Changes Caused by Chronic Nitrobenzene Exposure"

"The Morphology and Histochemistry of the Hemochorial Placentas of White Rats Following Nitrobenzene Poisoning of the Mother"

"Studies on Iron (Fe 59) Metabolism in Experimental Nitrobenzene Poisoning"

"Medico-legal Problems Posed by a Fatal Poisoning After Accidental Ingestion of Nitrobenzene"

Such literature establishes the hazards associated with nitrobenzene.

However, none of these articles provides simple, direct mortality information. We had to search a great deal of additional toxicity information to find the two LD_{50} and one TC_{50} in Figure 2 for nitrobenzene.

As a further example, the Water Quality Criteria Data Book, Volume 5, Effects of Chemicals on Aquatic Life, EPA Publication 18050-HLA, is an extensive compilation of data on the effects of chemicals on aquatic life extracted from literature published during the period 1968-1972. During that period only 170 articles by 138 authors were specifically on the effects of chemicals on aquatic life. For a group including all pesticides, PCB's, drugs and oil dispersants there were reports on 429 separate chemicals. All other chemicals totaled 161, or 27 percent. Of these 161 chemicals, only 50 percent had an associated TL_m value, averaging out to only 20 TL_m values reported per year. Thus, calculating extensions of those values as was done earlier is an effective use of these few values. Lacking that alternative, we must judiciously use the bioassay to incorporate the toxicity unit concept into permits.

There is an alternate approach to consider since it is difficult to determine TL_m 96-hour values, which involve the concept of toxicity, when in many cases effluents may not be toxic at discharge concentrations.

For effluents which fall into categories where toxicity is much less than 0.05 TU a direct flow-through survival test where some particular aquatic organism (presumably a fish) must survive in undiluted effluent for 96 hours is appropriate. Occasionally this effluent would

have to be diluted (generally in a range from 1 to 10) so that the fish would not be environmentally stressed. This would require the judgment of a professional biologist on a case-by-case basis. Survival for the 96-hour exposure period would be evidence that the discharge was not toxic under those conditions. Chemical analysis and limiting of the particular group of interest would then prevent degradation.

Although this approach avoids the use of toxicity, it does have the complication of introducing case-by-case determination.

Summing up, a method, the toxic unit concept, is proposed for applying toxicity control to wastewater permits. Data needed to apply this concept are limited; however, a method has been developed to extend the usefulness of these data. Two alternatives exist in that the bioassay, either TL_m 96-hour or 96-hour exposure survival, can always be substituted for calculation.

An article entitled "A Water Quality Index-Do We Dare?"[92] reviewed various attempts to establish a meaningful reference to water quality, stating that "With the current rate of environmental degradation improved procedures for ecological monitoring and environmental education must be developed." A methodology for toxicity was included in the development. "A Water Quality Index-Crashing the Psychological Barrier,"[93] by Robert M. Brown, President of the National Sanitation Foundation, drew attention to the need for a uniform yardstick for measuring water quality. While the use of toxic units attempts this, the concept will be viewed with valid skepticism by both industry and environmental toxicologists. Recognizing the assumptions and limitations

developed in this report, it is obvious that the use of toxic units is not an ultimate answer, but it more directly places the burden on the discharger to maintain a quality environment.

"And in such indexes, although small pricks to their subsequent volumes, there is seen the baby figure of the giant mass of things to come."

Shakespeare, "Troilus and Cressida," I, iii, 343, (1601-1603)

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The title of this Symposium is most appropriate i.e. "Structure Activity Correlations. . . . .". It is appropriate because it reflects the great need we have in Environmental Science to make predictions of what impact our present actions might have on the environment at some future point in time. It is further appropriate because it helps to negate the tendency of some experimentalists to laugh at theoreticians for having the audacity to make predictions. However, the fact remains that all of us, experimentalists or otherwise, use models to "explain" their research data and then predict what will happen in a different situation. For example, the relatively simple task of determining an acute LD_{50} uses a probability model to arrive at a number. Once the number is generated we are all aware of how the various regulatory agencies use this number to make predictions of what a safe level should be in our various water bodies.

Mathematical models then are simply a tool we all use in running particular experiments. These models can run in complexity as I have said from the simple probability model for calculating a LD_{50} to a very complex and sophisticated model to describe an entire Ecosystem. I am sure that people at this Institute are very aware of these latter models and have made significant contributions in their development. Another type of model is the one that Drs. Leo and Hansch along with Dr. Martin have developed in their use of statistics in making correlations of the structure of a drug with its biological response.

Our own use of models has been somewhere in between which has caused me to make the following analysis of model building. There appears to be at least two main purposes:

1. To help summarize data to help the decision maker in the following areas:

   (a) Interpret the data

   (b) Guide ongoing data collection efforts

   (c) Make predictions on future events

2. The second approach is to design and build a total mechanistic model of the Ecosystem that is of interest. Once such a model is built perturbations by new inputs can be made and the resulting consequences can be simulated.

One further general remark which is no doubt obvious but I feel is of sufficient importance to reiterate. Before embarking on any model building exercise it is very important to decide on exactly what questions are being asked and what information you want to generate. This is true of any project but especially relevant in this area where different diciplines are attempting to interact.

As members of the Chemical Industry we are continually faced with the problem of predicting the time/space distributions of our products in the environment. Once we have such knowledge the concentrations anticipated can be matched with a known toxicological properties. This type of problem has caused us to work in the area of compartmental models as a technique for describing the relations that exist between the various components of an Ecosystem.

Compartmental models have been used extensively by people in the pharmaceutical field and most of the theoretical discussions are given in that literature. Essentially we assume that various regions in the Ecosystem can be represented by a series of ideal volumes in which chemical substances move from one volume to the next according to the laws of kinetics. These ideal volumes imply that all property variations are ignored and perfect mixing is assumed so that the outflow from a compartment has the same properties as the compartment contents. We further assume that a single parameter is sufficient to characterize either the entry or the exit of a substance from a compartment. The Ecosystem of interest can then be characterized by connecting several compartments together. The notation and schematic representation of such a model is shown in the first slide. The parameters k_{ij} represent the transfer of a substance from the i^{th} to the j^{th} compartment by some mechanism which is left unspecified.

There are basically three different ways a substance may be introduced into an Ecosystem.

1. Bulk addition with no measurable time delay

2. Infusion over a measured period of time

3. A combination of 1 and 2

Variations in the amounts of materials present in the different compartments may be described by a system of linear first order differential equations.

The resulting equations may be either solved in a closed form or they may be solved numerically with the computer packages that are available for this type of problem.

Given any such system the problem of estimating the rate constants or the derived coefficients (i.e. the parameters) from measured data is a non-trivial task.

MODEL BUILDING

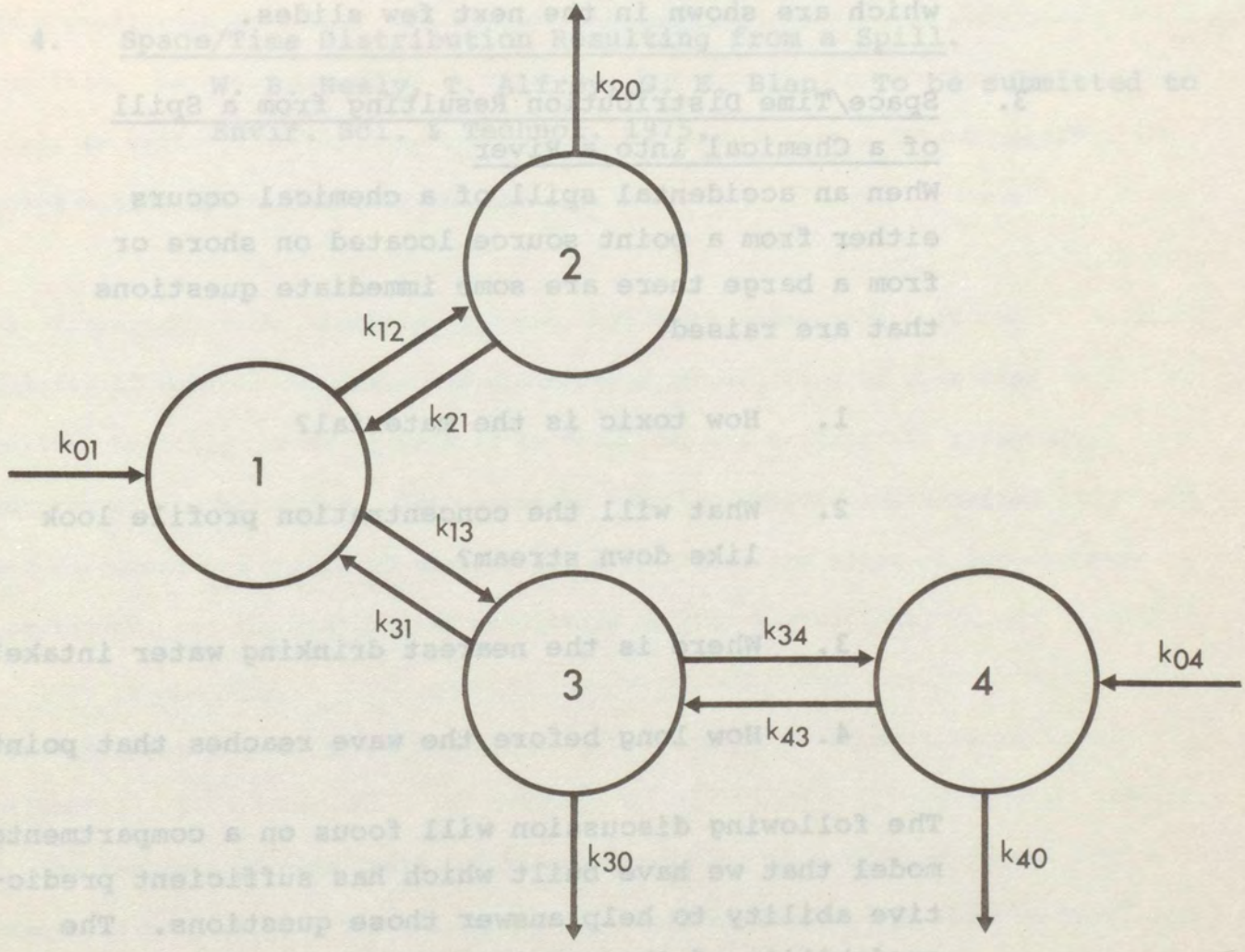
It is frequently possible for the ecologist to postulate several different compartmental models for characterizing the behavior of the substance added to the Ecosystem. An important problem is to decide which model is "best". Model discrimination is the statistical procedure used to help make this decision. Note that such discrimination takes place only among the postulated models. Residual analysis can help point out the inadequcies of the "best" model and help suggest improvements.

The steps in model building are summarized in the following slides. It is usually best to start with the simple model and proceed to the complex until no further complexity is warranted. This is referred to as the principal of parsimony.

EXAMPLES OF MODEL BUILDING

1. Bioconcentration - Dr. Branson has already discussed this example. Suffice to say that in order to apply partition coefficients as a predictive tool a model had to be designed to explain bioconcentration. Once the model was found to satisfy the data the use or partition coefficients was a natural extension.

Figure 1
A TYPICAL FOUR COMPARTMENT OPEN MODEL



We see that in spite of the complexities of the reactions involved a simple relationship can be established using this type of model building.

2. Distribution of an insecticide in an Ecosystem - Smith published some results on the distribution and fate of DURSBAN® when it was added to a model Ecosystem. By characterizing the data with a compartmental model it was possible to identify the important steps in the distribution. This type of analysis led to some important conclusions which are shown in the next few slides.

3. Space/Time Distribution Resulting from a Spill of a Chemical into a River

When an accidental spill of a chemical occurs either from a point source located on shore or from a barge there are some immediate questions that are raised.

1. How toxic is the material?

2. What will the concentration profile look like down stream?

3. Where is the nearest drinking water intake?

4. How long before the wave reaches that point?

The following discussion will focus on a compartmental model that we have built which has sufficient predictive ability to help answer those questions. The credability of the model is demonstrated by comparing the concentration profiles predicted with the actual profiles measured in two different spill incidents.

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DISCUSSIONS

CHAPTER 14

VEITH: We now come to the difficult task concerning the question "What can be expected of structure-activity methods in aquatic toxicity testing and bioaccumulation studies?" I have observed discouragement expressed by a few colleagues when they were informed that structure-activity correlation was a predictive tool only when appropriate compilations of data were available. We have emphasized at this workshop that the correlations are models to systematize thinking and forecasting rather than a "black magic" method producing a prediction of toxicity.

Dr's. Kopperman, Zitko, Kimerle, Branson, and Macek have shown that the toxicity of related chemicals and the residue accumulation of even dissimilar chemicals can be correlated by selecting the appropriate structural features of the molecules. The papers by Dr's. Leo, Martin and Schaffer have discussed the nature of some of the parameters, the types of correlations experienced, and the statistical properties of the observations.

Thus, the potential of these predictive methods might be summarized as follows:

1. The residues of many lipid soluble chemicals may be estimated from structural properties. However, there is a need to account for the outliers and standardize bioaccumulation methodology. Also the possible confusion caused by the use of radioactive chemicals must be noted.

2. The relative toxicity of organic chemicals can be related to the structural variations of the series of chemicals. However, Drs. Martin, Zitko and Gardner have pointed out the very important problem that the end point used in the correlation must be clearly specified. Moreover, data bases of appropriate parameters must be made available to the research community.

These comments may summarize the two main topics of concern; i.e. bioaccumulation and toxicity of industrial pollutants in the aquatic environment. However, there is an even larger problem in the area of applying laboratory data of this nature to bioaccumulation in the environment and water quality objectives where mixtures of chemicals are present. How can these data be related to field observations? Drs. deFreitas and Neely have shown some very practical considerations which must be accounted if regulatory agencies hope to apply laboratory measurements to field situations.

Moreover, the toxicity estimates of individual chemicals from correlations with related chemicals can only be useful if the toxicity of mixtures can be modeled. Drs. Anderson and Seba have discussed several different approaches that might be used in the problems of the toxicity of mixtures. We also are faced with the problems of spilled chemicals or periodic exposures in mixing zones which cannot yet be modeled by current methods. It seems unlikely that we can

ever predict the possible effects of periodic exposures unless the uptake and clearance of the chemical can be estimated.

At this time, the workshop will provide a panel discussion of the problems which have been identified and those submitted in writing by the observers of the workshop.

A. General Panel Discussion

VEITH: The first question is by Dr. Chow to Dr. Anderson. "Have you considered the complexing capacity of the medium as a parameter in the models for toxicity of copper and nickel? We have found that the toxicity of metals is closely related to the complexing capacity of the medium."

ANDERSON: Our test system didn't take into consideration, as far as the assaying is concerned, the ratio of free copper to complex copper in the test chambers. We assumed that there was some direct relationship, however, between the total copper and the total nickel that were assayed and the toxicity which we observed under the standard conditions held throughout the 96 hours of the experiment. We were aware that the organic material in the water will chelate the copper and nickel forming complexes; however, we would like to point out that one of the more effective chelating agents in our system may be the gill epithelium of the fish. Thus the copper or nickel may be affiliating with the ligands of the proteins of the gill epithelium. We even in fact hinted that gill epithelium may be the major site of the lethal action of the heavy metals.

VEITH: Many of the questions that have been submitted have the same theme in that the authors question the value and application of a tool such as structure-

activity correlations. I have a question for Ken Macek. "Having studied the bioaccumulation of some 50 compounds, what is the utility of the bioaccumulation data from clean water lab systems using intact compounds? What about compounds that are degraded in the environment and/or by the organism?"

MACEK: The objectives of looking at the 50 pesticides were to support pesticide registration and to satisfy the requirements of the Pesticide Regulations Division of the EPA. My understanding of the rationale is that these data are strictly a mechanism for identifying in their permit process those compounds which, in relation to other compounds similarly studied and similarly utilized in the environment, represent the greatest hazards to the environment. Also, the data identifies those compounds which may require a more indepth evaluation of the relationship between toxicity and hazards of the compounds and the use in the environment. In response to how we relate this to the residues that occur in the environment from the use of any particular pesticide, we now are trying, in a relative sense, to evaluate this hazard in a more realistic manner by allowing some of those processes which do occur in the environment to occur in our laboratory systems and to assess the impact those processes have on the bioconcentration factor. Very clearly, for those few compounds that we have had the opportunity to study in both the fish/water laboratory system where the parent chemical concentration is maintained continuously and a system where we allow some of these other processes to occur, we have seen very dramatic and divergent results with respect to the finite residues, the absolute amount of

residues present, and the relationship between concentrations in fish versus concentration in water. They display different pictures of what is going on. We recognize the limitations of the fish/water system and the fact that it is relative as is the second case. However, the second case, although relative, is a little more meaningful.

VEITH: Dean Branson - would you like to comment on this same topic?

BRANSON: It seems like one of the questions on our minds is assessing the true hazard of pesticides. Placing the chemical in a simulated static water system containing soil, letting it age in the presence of fish, and examining the residues accumulated does give us a yes-no, hazardous-non-hazardous categorization and may allow classification of chemicals that way. On the other hand, if the question is to assess the hazards of a particular concentration of a chemical, then the static test falls short. Then the first bioaccumulation test that you described would be more valuable. The relationship between the concentrations in the water and the hazards of the residues in the fish is the more important question.

MACEK: The way one would determine a "bioconcentration factor" is really dependant upon the objective one has in utilizing the "bioconcentration factor" concept.

There are a number of groups addressing the question of how one determines what methodology should evolve for determining bioconcentration factors. One of the first questions to be answered is what are they going to be used for and by whom. I'm not at all optimistic that a single, methodology standardized for determining bioconcentration factors will be adequate for all the purposes for which people might wish to put the concept to use.

SEBA: I think some comments on this question should be made here from U.S. EPA because it bears on some of the rationale of the Public Law 92-500. What that law is saying - why we have a permanent program for effluents is that, even though we really don't know what is going on in the environment, we can control the pipe before it goes into the environment. Therefore, the type of testing that is done on the effluent gives us a clue whether we should put some limits on what is coming out of the pipe. However, for example, if we consider the problems of chemical spills, the law has an entirely different approach. We're struggling with another type of legislation to approach the problem of accidental spills and determining the effect on the environment. Let me give another aspect. If a chemical is felt to be highly potent and should not be released into the environment, there is yet another mechanism. Nonetheless, the biggest pollution problem that we have comes from discharges from municipal and industrial practices. The intended law today is directed to controlling the pipe rather than water quality criteria, i.e. rather than looking at assimilative capacity of the water. That's gone by the boards now because we just don't have a handle on assimilative capacity so we must try to control it where we do have a handle.

BRANSON: Are the criteria the first objective and then the calculations for the end of the pipe rather than the other way around?

SEBA: There is a provision where the water quality limitation I mentioned will be applicable. That's where there are many discharges and we have to be stricter than we would be normally. In other words, if there was a lone discharge in Lake Erie, we could look just at data developed in the laboratory in arriving at a suitable permit. However, if ten industries were right next door to each other where there is poor water circulation, you must just overwhelm that body of water and we would have to be more restrictive. The law only deals with water quality in that sense and we are concerned about water quality only in those cases in the permit program.

KIMERLE: I would like to re-enforce the point that Ken was making. I believe there are two distinct types of bioaccumulation tests that we should be interested in, especially with respect to methodology development and the critical question of the purpose of the test. If we study the bioaccumulation of an interesting homologous series of compounds to determine if there is a relationship, then clean water systems using intact molecules are a must. However, if we are evaluating environmental hazards, we must use one of two tests; one that has an intact

molecule tested in a clean system and the other which examines the fate and metabolites that may occur in the environment in an attempt to evaluate the bioaccumulation potential of all of them. This is often completely different from our experience.

VEITH: In considering a protocol for the testing of new chemicals, of which I understand there are in excess of 500 a year, it is likely they will have to be screened by a number of methods. Thus, there is a tremendous burden to do a substantial amount of testing each year if we pull back from all the nitty-gritty of all the research details and look at the chemicals that are present as residues in the major bodies of water. In my work, we have been working with the Great Lakes and some of the major river systems through exploratory studies, concerning ourselves with what chemicals are present in fish at any concentration rather than effects. The chemicals that generally keep showing up most often are those that have partition coefficients in excess of 10^3 or perhaps 10^4. This is not a cause and effect relationship, i.e. you cannot set a guideline that says any chemical that has a high partition coefficient is going to be a problem because the tonnage produced and the use must be known. However, whether or not a chemical has a high partition coefficient might be very important information in a protocol for raising a red flag indicating a need for further testing. There are many compounds that have large partition coefficients that may be metabolized and the bioaccumulation test is an essential part of a testing program. I think there was a slide on the board that showed many of the chlorinated

hydrocarbons and other persistent industrial chemicals that are found in the environment have high bioconcentration factors in fish and have high partition coefficients, or lipid solubility. Developing a method which could empirically throw up a red flag in the screening of new chemicals could be an extremely important part in correlating a structure like this to use of the chemical.

VEITH: We have a number of questions concerning this topic. This question is for any speaker on the bioaccumulation of organics. The chlorinated hydrocarbon contamination of Lake Michigan coho salmon appears most closely related to residues in the food such as alewives than the residues in the water - how do the lab studies of accumulation of contaminants in water relate to the observed field situations? There is no name on this question. Does anyone want to discuss this?

DE FREITAS: The question really seems to be which vector is the important vector in the coho salmon, whether it will be water or food. I don't think a generalization can be made unless specific information such as the water concentration and food concentration of the fat soluble pollutant is available. Our studies suggest that under a steady state situation, 90 percent of the accumulated body burden can be derived from the water factor at least as far as pollutants of DDT types are concerned. But again, there is no magic answer as to water or food factor.

HAMELINK: About this question of what is more important - the uptake from the food or uptake from water, we are just finishing three years of work on a large-scale model system. The uptake efficiency from DDE in a model system is incredibly efficient. You just have to give that fish credit for picking almost everything that comes to him by his food and by his water. That is really not the issue. The better question is, how well does this animal retain? We keep talking about uptake, uptake. But retention mechanisms become even more interesting and is something I think we have always tended to neglect. How do they retain so much? The second point that always worries me is that most people do not understand the difference between an uptake efficiency, i.e., the percent extracted by passage over the gills is not necessarily the same as its bioaccumulation factor. We tend to make the assumption that because it is efficiently taken out of the water, it has a high bioconcentration factor. It's nice to know it, but unless you know rate constants and how fast it passes across the gills, you may be deceived. Probably the compound with the highest uptake efficiency will be oxygen in water, but it is not bioaccumulated. Don't let that rapid uptake curve deceive you into believing that it is bioaccumulated at high levels.

BRANSON: Recently, Gruber et al., in the February issue of 1975 Environmental Science and Technology discussed the accumulation of PCB isomers in juvenile coho salmon - accumulation measured by dietary studies. Moreover, there was a dietary study by Leeve et al., in the Journal of Agriculture & Food Chemistry in 1974.

One study was at approximately 10 ppm in the diet and the other used 15 ppm in the diet. In neither case did the concentration in the whole fish, whether it was a trout or a salmon, acquire the concentration found in the diet. So it seems to answer the question that was raised about alewives and coho salmon in that the coho salmon eating the alewife that has 10 ppm would not likely acquire 10 ppm as a body burden. However, in fact they do get much higher than that.

HAMELINK: Did they have clean water or did it have a concentration of PCBs?

BRANSON: No, it was clean water.

HAMELINK: You can't keep it that way.

BRANSON: These papers both support zero elimination. They put them in fresh water after exposure and their clearance phenomena is very similar whether they were exposed to contaminated water or diets.

ZITKO: We observed the same with Atlantic salmon. Once we take them off the contaminated diet, there is growth dilution by which the concentration goes down but total body burden is constant. There is no clearance whatsoever.

BRANSON: I think I should elaborate on a point that I didn't make in my presentation - we define clearance in our kinetics on a basis of concentration. If we define clearance on the basis of chemical for the total fish, the growth dilution probably would have cut the rate of clearance down by half. We did see some clearance but it would have meant a halflife of 60 days instead of 30 days.

DE FREITAS: If you take growth into account, you need an assimilation efficiency at least ten percent or maybe up to 20 through the gut from the food in order to see any indication of a bioconcentration effect from the food vector because in a rapidly growing organism, you can usually figure on ten percent of the dietary intake will result in increased body tissue. You can't expect a bioaccumulation (an observed increase in concentration) unless assimilation efficiency through the gut is greater than food conversion efficiency for growth.

VEITH: I have two related questions here. The first part - from Dr. Kimerle to anyone. "The title of the workshop was predictive toxicology - predictive of what and how can we correlate things?" Similarly from W. Strachan, CCIW, "The purpose of this symposium was to determine whether there was any feasibility to predicting toxicity of organic compounds based on structure considerations. The partitioning or accumulation approaches may be useful for predicting tissue levels but does not address the question of toxicity. There has not been any address to predictions of toxicity which involves the different functionality

in the examined compound. Would the symposium participants agree that it is not possible to theoretically predict toxicity of organic compounds based on structure and/or physical chemical properties alone?" Perhaps Dr. Leo or Dr. Martin could address that question.

MARTIN: Of course, this is the question we in the drug industry consider as well. There are a multitude of ways to get to the same effect and it is difficult to expect that one equation can describe the lump sum. I would say that it would really be a pipe dream to think that we could just look at a structure and say whether it will be toxic or that it's not. But that does not mean that it's not valuable to collect information and examine patterns and relationships within the data. It's a lot more expensive to collect the data on the individual compound than it is to think about what those all mean. Certainly, computers and people like myself are pretty cheap compared to doing long term toxicology type studies. I think we have to keep it in balance.

VEITH: I'm really glad this question came up because I think that the brief title of the workshop was misleading. One of the major considerations concerning the Hansch approach, or related approaches, is that it is a prediction or an estimate resulting from a data base - on empirical estimations, and not generating numbers out of the blue. The interesting thing that has come out of so many of the studies is that, when there are data bases to work from, the data starts suggesting useful patterns and modes of action that wouldn't be seen if you just started randomly testing chemicals or examining data.

MARTIN: One important concept that has come out of the Hansch approach is the idea of optimum partition coefficients. For example, within a homologous series, there may be one molecule which best penetrates the brain. This optimum partition coefficient is often the same for different series of compounds even though the series might have different intrinsic activities at the optimum. This is seen with penetration to the brain after intravenous administration and with antibacterial activity with different classes of compounds. It is these patterns we should look for so that we could obtain information on a few members of a series to estimate if it will be worse or better. As we test each one we could see how that fits within a framework, not just collecting data and putting it somewhere. We are trying to get a little more out of it to help us design our next experiment.

VEITH: Dr. Kimerle, could you answer or comment on your own question.

KIMERLE: Well, I think I did on my first comment. There is a need for two types of tests. It depends upon what questions are being asked about the test, i.e., whether you are trying to predict environmental hazards or attempting to generate correlations for pharmaceutical reasons. We should be well aware of what we are trying to predict. If it is environmental hazards, we should attempt to put the data in prospective of what is happening in the environment.

BRANSON: I would like to underline some of Yvonne Martin's comments concerning structure-activity relationships within homologous series. The chemical industry and the drug industry are trying to find particularly useful compounds that are

going to be commercialized at the same time that a group such as Dr. Macek's received the sample for his fish studies. As Dr. Zitko mentioned, there has usually been a homologous series studied and one compound selected for commercialization which is the optimum in terms of efficacy. Today, efficacy alone is no longer the only criteria for selecting the optimum homologous series. Rather, toxicity and bioconcentration potential are becoming more and more important. Our studies of a homologous series consider the structure versus what would be the predicted toxicity or predicted bioaccumulation. If there are two or three members of a series and we have rough data on them, it is amazing how often you can arrive at the best member within a homologous series from these rough calculations.

MACEK: The biggest potential for structure-activity correlations in the regulatory agencies, the industries and the consulting testing organizations is that currently there is no way all the necessary information can be generated on each chemical that is used, considered for use or has been used. If structure-activity correlations can assist in establishing priorities or even providing a reasonable basis for eliminating compounds from categories, that itself is a worthwhile objective and probably is one of the first productive results that can come from structure-activity correlations.

MARTIN: I will emphasize that one of the things that has come from drug studies of the Hansch type approach is the "rational design" of a series of compounds. It is certainly well documented that within a series, it is not necessary to

make and test all of them. If you design properly and do the test well, you can get as much information from ten compounds as from a hundred. This applies to toxicity testing as well, providing the chemicals have the same mode of toxic action. The idea is to test as diverse a group as possible but without major differences in the mode of action between them so interpolations rather than extrapolations can be made.

LEO: In an idealized case, you might have either a pesticide or herbicide that has a well defined equation with three significant parameters which indicate which ones are the most advantageous product. If one of these is log P which seems to be quite directly related to bioaccumulation and another an electronic factor, perhaps you could dwell on substituents that would lead you to the optimum through the electronic factor which might even lower log P and hopefully lower bioaccumulation. In other words, if you had a positive coefficient with both σ and π instead of the most lipophilic with the desired activity, you might use the most electronically effective which may have a lower log P and hopefully a lower bioaccumulation factor.

KIMERLE: I would like to make a comment on Ken's (Macek) point of singling out and finding the bad apples using an ecosystem approach. The four facts that Dr. Branson brought up--toxicology, bioaccumulation, stability, and mobility of the molecule in the environment must be examined.

SEBA: Perhaps I didn't make the point clear in my presentation. The comment that we started with was that there was no discussion on structural-activity forecasting. I have a number of references to that in my paper, particularly taken from computer programs used in the drug industry to forecast toxicity. It is a very elaborate thing as Yvonne (Martin) mentioned. What I was proposing was, by using something such as the coefficient of relative potency for 14 different classes of phenolic compounds, i.e. alcohols, nitrates, etc., I was able to forecast a TLM 96 hr. within less than an order of magnitude. Now, it's not earthshaking, but it is a utilitarian number that we could use in the toxicity-unit concept, and we need something like this in EPA. I welcome any comments either on the toxicity-unit concepts or using something like that. I feel a little outgunned by my colleagues here who are dealing with sophisticated models and worrying about fitting everything into their boxes. I'm just worried about trying to come up with a rational number that I can use in a permit on an effluent. There may be promise in using something of the nature of the coefficient of relative of potency. It works for 14 different classes of phenolic compounds and it may work for 14 hundred or 14 thousand of them for our purposes.

MARTIN: I would like to comment on that. I think it should be clear that you have to think about interactions, especially two compounds being more toxic together than estimated from the toxicity of the individual chemicals. By your calculations, you are assuming the toxicities are additive. Maybe that is the best you can do, but data more of the type Dr. Anderson talked about, including interactions, should be generated.

AMSON: Let me comment on what Doug (Seba) said and let me raise several points. We seem to be talking around the subject at hand and I'm referring to what Gil (Veith) raised a moment ago with regard to what predictions have we seen in the last few days, i.e., what abilities we have to predict? I'm bothered, speaking as a person who is in the legislative-regulatory field, by the inability of the researchers and the poor-sons-of-a-guns like me who have to write the law to mesh. When you put together a model, it's fine if it holds true. Let me give you an example of one that did not. I am sure most of you are vaguely familiar with the famous water law - Public Law 92-500. As you probably know, there are a great number of sections to that law. One of them deals with a permit authority that Doug is concerned with, one of them deals with water quality criteria that has been referred to under Section 304-A. The point I wish to make is in regard to Section 307 and then I want to go on to another section from there. Section 307 says in effect, "there are a few substances that are so toxic that their discharge ought to be totally banned". It goes on from there to say "find out what they are and give us the levels at which they should be banned". We became involved in it over a period of time and we tried to solve the problem from a hydrodynamic model. We talked to the people we presumed knew a lot about hydrologic models, and we rapidly found that for specific waterways such as at Vicksberg, Mississippi, the Corps of Engineers does a superb job of modeling a particular estuary or a particular river between two points. But applying that model to another estuary which may be next door doesn't work (expletive deleted). Because the model is so exact, it can't be generalized. We

ended up with four very bad models: one for a river, one for a stream, one for a lake, and one for an estuary; actually five - one for coastal waters. They're not really very good but they're all we've got. Let me go on to the third section. Section 311 deals with hazardous materials and the problem that Doug referred to, those of spills, extra-ordinary incidences such as when a railway car goes off the track and a chemical is spilled or a tanker truck rolls off the highway. There are some 375 different substances on that list in the advance notice of proposed ruling. Let me come full circle now, back to predictive toxicology. There are four points to that Section 311. One of them says "designate them, what are they"? This is where the 375 chemicals come in. The second one says "tell us what constitutes a hazardous quantity". A tank car is leaking a drop an hour of this substance--if that doesn't constitute a hazardous quantity, what does? The third part says "tell us what the penalty should be for spilling it" bearing in mind that the penalty is not the same for any of the 375 substances. It just has to be "in a common unit of measure". The fourth clause says "tell us if it is removable or not"? because the penalty is different whether it is removable or not removable. I don't want to get into the legal/economic hassel that's involved here, but let me say that the possible penalties under Sections 311 are huge. For example, if a vessel carrying a hazardous material spills it in a hazardous quantity, it is possible to fine up to $8,000,000. If the insurance company refuses to carry insurance for $8,000,000 the vessel will refuse to carry, and there will be many other ramifications.

Let me get back to where I was. If we have to define hazardous quantities and write the penalty regulations for 375 different substances, we'll be doing it from now to the year 2000. Isn't there a simple way, without determining partition coefficient and parameters that Doug suggested, to predict on the basis of not so much of toxicity (because we have information on 10 or 20 or at maybe even 30 percent chemicals on the list) but on the basis of some innate physical-chemical-structural properties of the substances themselves? Are we really at the point that we cannot do a prediction of hazardous or toxic materials? Are we really back at step one and a half?

MARTIN: Is it really true there is absolutely no information on the toxicity of these 375 substances?

AMSON: No, let me say for instances of the 375 substances I'll be willing to bet that for 20 percent of it, 96 hour TLM data on a pretty wide range of organisms exists. The other 70 or 80 percent we don't. The point I was raising was, since they fall into chemical groupings (there are ammonia compounds, there are chlorides, nitrile groups), isn't it possible to know something about the chemistry of one of those members to set a hazardous quantity, a rate of penalty for all the other members in that individual grouping. If I could make ten groupings of 37 chemicals exactly, rather than one times 374, it makes the job ten times easier.

MARTIN: I don't see why you don't get the manufacturer to provide you with the information that is required? Why should we as citizens pay for determining what the manufacturers are doing. They are putting the profits in their pocket, so why don't they supply the information?

AMSON: Unless I misunderstood you, a great number of the substances are not drugs--they are chemicals.

MARTIN: No. However, if I want to sell a drug, I have to prove it's safe. If companies want to ship something on the Mississippi, why don't they have to prove it's safe.

AMSON: Well, let me answer that two ways. The shipping regulation itself already exists. DOT has rather complicated regulations for transporting materials. Further, if they have to prove starting now that there is no hazard, then we are back maybe not to the year 2000 but the year 1985, because these things are being transported daily in massive amounts. We are trying to come up with regulations that will say what the relative hazards of shipping and spilling are, in the next couple of months.

VEITH: The questions that I have left are really merging into "what good are these predictive tools?" It is my feeling that predictions of toxicity will

never be sufficient from a legal viewpoint. At the same time, it's my feeling that 96 hour LC_{50} data exists or can be quickly generated to predict the toxicity of structurally related compounds precisely enough to be extremely useful to any enforcement agency dealing with effluent restrictions. The problem with making an estimate is that the next step is to defend that to a $300-a-day attorney, and I just don't see how predictive toxicology structure-activity correlations will ever replace detailed water quality or toxicological studies. If there is going to be a restriction set on a compound, the test will have to be performed. However, the models are essential to estimating the toxicity of large numbers of chemicals in order to set priorities for study.

For example, there are a number of groups in the world that are analyzing chemicals in effluents. A few years ago, an organic characterization program had identified 275 chemicals in industrial effluents. The concern was "when these lists become known, we are going to have to know something about the toxicity of these chemicals" We were able to select 30 chemicals of eight major classes from this list to start testing to try to get some feel for the toxicity of each. Within six months EPA had bought more than 20 mass spectrometers. Now the list of chemicals is in the thousands. We are not going to be able to test all these chemicals and we're going to have to design an acceptable data base for making estimates. The structure-activity approach is the best method to estimate toxicity; however, incorporating the estimates into the toxicity-unit concept is a second problem.

MACEK: If we have toxicity information on aquatic organisms to the extent that Dr. Leo has information on partitioning coefficients, we could look at the data base and make the conclusions with some reasonable degree of certitude and that job would be a lot easier right now. Unfortunately, we are several years away from that. Somebody must get on the horse fast and decide what it is that we need to do and the best method of doing it.

LEO: Where the toxicity is specific, we can tell what the alcohols are going to do with proportion to log P, but where toxicity is non-specific, this grouping becomes difficult.

AMSON: You hit the nail on the head. Ken is quite right, if we had a hundred million dollars, in ten years we could give you an answer. But we don't have ten years and whether we have the money is not the point. Dr. Leo just hit it, if we have non-specific toxicity where do we go?

VEITH: I don't think it will take that long or cost that much. We will have the same questions in three years. If we start now, we can construct a systematic approach.

HAMELINK: We've talked about partition coefficients, toxic effects and bioaccumulation. We are interested in what controls the concentration in the water, in the environment, in addition to what controls it in the animal.

SEBA: If predicting toxicity is risky, then making a political future of prediction is even more risky. Your comment was that if we don't get around to testing all compounds, we are not going to have a legal handle. My comment is that after 15 years in that area of environmental regulation, what seems to be happening is that if an environmentalist said to somebody 15 years ago, "all that stuff that's going in the water is not good for the fish", the reply was: "oh really?" The reply has shifted in more recent times to "well, you've got to prove it to me first". Congress has concluded yes, it is bad for the organisms and we are turning to you, EPA, to come up with a way to regulate this. EPA has run into a resistance particularly from the industrial community. When we find a particular industry with their particular problem, they can resort to the legal conflicts of proving harm. Perhaps we will get to the point where industry will adopt the philosophy you have mentioned. Some of the proposed bills will require industry to prove the safety of each and every substance. Congress is saying "okay we won't deal with water quality, we'll deal with the pipe and put the burden bit by bit on the polluter. However, we are at a point that we must deal with large volumes of chemicals and chemical types or we are going to find ourselves at an ever increasing regulatory situation of trying to pin down each and every chemical.

NEELY: I would like to make a comment since the issue of Section 311 has been raised. This Section deals with spills of hazardous chemicals in harmful quantities. It really raises the point that predictive toxicology is many things. What is it you are trying to predict--LC_{50}, carcinogenicity, teratology? Harmful or hazardous quantity is even worse to predict. What do you mean by hazardous?

I think EPA recognized this problem and contracted the problems to Battelle who wrestled with it for three months. Battelle has done a tremendous job in trying to answer these very difficult questions raised by Section 311. One of the ways Battelle tried to quantitate this hazardous quantity and, in essence predict it, was to set up a matrix of seven or eight hazardous properties. Then they listed all the physical and chemical properties they could think of, i.e., does the chemical float, sink, etc. They sent this out to many different people and had then answer the questions by the Delphi approach. I received this questionnaire and had to put down a number from one to zero for each question. I guess they have to repeat this several times until they come up with a concensus concerning the most harmful or hazardous. Until something better comes along, this isn't a bad idea. Now I'm not sure what the gentlemen of EPA are doing about the Battelle report. It has been recycled several times now and I think they're due to regurgitate once more. I don't know if they are going to use the Battelle report as a basis for their ability to write regulations under Section 311 or not. I do think the Battelle approach is a good concept. There are some problems with it but this is as close as anything I've seen to predicting a hazardous quantity. I don't think we're going to come up with predictive toxicology as they do in the drug industry.

AMSON: Battelle was contracted to come up with at least three approaches. EPA gave them three potential ones and they came up with three of their own. The Delphi approach was one aspect of it. The work Battelle did will be a substantial part of what ultimately will become 311. We simply took those four

volumes of the data report and hybridized the approaches to produce two more which will be a major part of Section 311.

VEITH: One thing that has come out thus far is that partition coefficients and other structural features (water solubilities, the pK's of ionizable compounds, some of the other energy terms, steric factors) are all needed to apply structure-activity correlations. However, not everyone who attempts to apply this technique is going to calculate these parameters. It is important to know where data bases are for this information and methods involved. Al (Leo) or Yvonne (Martin) could comment on where data bases are and a little on methods of determining them.

LEO: I'd like to comment on data that we're connected with at Pomona College. Our medicinal chemistry project attempted to collect some pertinent physical-chemical parameters in measuring log P's in octanol-water systems as well as a few other systems. We are beginning to measure some parameters and attempting to screen the literature for these values which are of significance in the bio-chemical, bio-medical field. We put this in a computer so we can organize a report every six months. We welcome inquiries concerning methodology and we have had visiting scientists at the college who pick up what they need to know in a week or as long as necessary. The amount of help we're willing to put out on this is about as much as people are willing to accept. Specifically, if anyone has use for physical-chemical parameters, they should try to receive this big voluminus data base by tape or by hand copy. I would be glad to send out

samples to familiarize one with what is available in terms of measurement.

It sounds so simple to measure a partition coefficient. You have two immisible solvents and you put the solutes and measure the amount in each phase. It doesn't really matter if you measure both phases because the ratio of concentrations is important anyway. What could be more simple? However, the n-octanol/water phase has been worked with most frequently and contains about two molar water when saturated. Also, there is a very small amount of octanol in the water. There is a certain amount of polarity in the lipid phase which mimics the amount of polarity of membranes in biosystems. We tend to ignore the amount of octanol in the water phase but we are finding instances where this is terribly important. Also important is the fact that, as you shake these things together, the octanol phase can get super-saturated with water. Normally we recommend centrifuging, but it's readily demonstratable that you can centrifuge many times gravity for 15 minutes and still not bring this excess water out of the octanol phase. The procedure is terribly important in that orders of magnitude errors are possible if you have too much water in the octanol phase. With ionic materials there are undoubtably a number of problems in interpretation. You have to remember that you are partitioning into the lipid phase in ion pairs and a mass action principle is involved. You have to either extrapolate back to infinite dilution, or specify the conditions. We are using sodium as a cation.

MARTIN: It has been in our experience with lipophilic compounds, it takes as much as a half hour with medium speed shaking (not so much as to get an emulsion)

to come to equilibrium. We put the thing on as soon in the morning as possible and do not analyze it until lunch time so that we are sure the phases are really at equilibrium.

LEO: That's the simplest mistake that we make. Actually, diffusion takes place quite rapidly and it's best not to overshake them. For surfactants shaking is a problem and the best thing is a rocking devise that can just exchange the surface area of the two phases. You'll come to 95 percent plus to equilibrium in a couple of hours and you don't nearly have as much trouble separating phases.

ANDERSON: Assuming that we are not going to be able to stop the problem where it begins at the pipe and contamination of water will continue, I think there is no other way than to do a full workup on each and every possible chemical contaminant. That's obviously an impossibility in the time span allotted in view of the numbers of chemicals and the personnel, money and the technology available. But I think that it has to be done in the long run; there's no other way. For only the total workup on each and every contaminant would allow you to get the safe limits for which you as a policy maker are searching to assure protection of the ecosystem. What purpose then do we have as modellers of toxicity?

In view of the number of toxicants that would have to be examined, we serve the purpose of directing where our emphasis should be placed. Models serve to isolate or identify series of chemicals or specific chemicals that represent particular hazards in the aquatic environment.

In relationship to the workup on the toxicity of each and every particular chemical contaminant, we should consider two points of view, the kinetic and the dynamic actions of chemicals in organisms. The kinetics involve those processes in the uptake, the accumulation and the elimination of the chemical contaminant whereas the dynamics involve the mode of action, i.e. the relationship between the chemical and receptor or receptors within the cell. Now, models which predict the characteristics of the kinetics of a particular chemical or series of chemicals do not necessarily predict the dynamic properties of the particular chemical or series of chemicals. For instance partition coefficients and structural correlations may have highly predictive value in relationship to uptake and accumulation of chemical contaminants (and in this respect they may be potentially hazardous) but we must not assume that such parameters are necessarily correlated with the dynamics of chemical contaminants. Consequently the toxicity (i.e. the dynamics) must always be investigated.

LEO: But partition coefficient models at least help us set priorities.

ANDERSON: That's the function I think we serve.

AMSON: That's the point I was really going to make. The regulators don't do the research; we depend upon you, the experimentalists, to come up with the answer. The real point as you expressed yourself quite well is that we ought to test all those dangerous things out there; however, which of the ones should we test first? That's the sort of thing that Gil and his people need to know.

Which of those are in the top ten and which are in the bottom ten, if there are a bottom ten. I agree with you 104 percent about its dynamics and kinetics and everything else. However, if kinetics don't help in getting more information about toxicity, then I turn to you as a research investigator and I say "what's a better parameter?" I don't know. If there is one, we'd like to know.

ANDERSON: I don't know if you can look at it from such a singular point of view, i.e. to ask for the better or ultimate or all encompassing parameter. I think that kinetics is simply one parameter, an important parameter but only one of many which should be considered. Obviously accumulation in an ecosystem is a primary consideration. We all know the experiences with DDT and other chlorinated hydrocarbons. Maybe some chemical contaminants that do accumulate are not toxic and vice versa that some chemicals which are toxic do not accumulate. One can not assume having studied the kinetics that one can predict the toxicity. Many parameters are therefore needed to properly evaluate the hazard which a chemical contaminant represents to the environment.

AMSON: That is true and let me refer back to something that we discussed yesterday afternoon. DDT is a good example because we were looking at it from the wrong angle all the while. It wasn't so much later that we found about the egg-shell problems. The investigators were looking at it from other points of view, then all of a sudden over a period of 3-4-5 years, it became evident it was dangerous from a totally different point of view. Going back to where you were a moment ago, if kinetics is important and if partition coefficients are important,

what are the parameters that we should be looking at and what about parameters that are the most important in order to set, for example, hazardous quantities?

ZITKO: You will never be able to test the toxicity of all the chemicals in the environment. It is impossible. First of all, it would take a long time to find out what we are putting in and even if we knew that, we wouldn't be able to do it. Structure-activity relationships can help us a great deal. For example, many organophosphate pesticides have several isomers which are also added to the environment. If we established the toxicity relationship for one of these, we can assume with a great deal of probability that we are being correct about the toxicity of the related pesticides. If we establish the toxicity of s-methyl isomers or s-ethyl isomers of a variety of organo-phosphates, we can make a conclusion about all of these things. This is one point I wanted to make. The other is we shouldn't place an undue emphasis on accumulation in the environment. You need to kill fish only once to do the damage and organophosphates or other non-persistent compounds may do the job, and we may miss them completely because we are looking at different things.

SEBA: I wish to follow up on your comments that we can't measure all chemicals. The 374 chemicals may not seem like too much, but there are over 50,000 compounds on the NIOSH Toxic Substance List. Moreover, we have about 50,000 permits and that covers a multitude of out-falls. Somewhere amongst those out-falls must be every one of those chemicals, or darn near every one of them. Right now, there is no mechanism on the permits for organic substances. We all

recognize the toxic substances; however, we need to evaluate many unknowns that are being discharged, and we don't have a rationale to put them on the permit yet. We recognize it should be there. We are charged with doing it and something has to be done at this point.

HALL: What were the criteria used for selecting the 374 compounds on the list?

AMSON: Some very specific selection criteria included factors such as it had to have a certain toxicity, it had to be produced in greater than research quantities and it had to have a reasonable potential for being spilled. There are also a number of other minor and major characteristics that had to be met. We started out with a great deal more than 374 candidates and this is not necessarily a final list. This is what is known as an advanced notice of proposed ruling. We still have to go to a proposed ruling and a final ruling and the number may change.

NEELY: I'd like to comment on the criteria that were used. From the data available on the 374, it looks as though the 96 hour TLM for fish was the main criteria and about 90 percent of the 374 chemicals have a 96 hour TLM value of 500 mg/l or less. Battelle came up with the same notion after considering all the characteristics that may cause harm to the environment from a spill situation. It's the fish toxicity that is critical.

MARTIN: What about the other 49,000 compounds which weren't tested? We don't know their toxicity.

HALL: I guess my comment was more of a probe than a real question. From this group of compounds which have already been selected as potential hazards, we have some basic information which could be used in a predictive way to categorize some of these. I remain unconvinced that we haven't got something useful. We have at least one common measurement for all these 374 compounds--can we possibly move from that?

LEO: If we examine these compounds carefully, we can pick out a number that do fall into the same kind of non-specific toxicity that alcohols do. In other words, all the alcohols, perhaps butyl acetate and a number of others may fit the same equation that Hansch and Dunn had worked out for the alcohols. It was for narcosis but a bit above narcosis is toxicity to tadpoles, fish, etc. I think you could pick out 20 from this list and predict the toxicity of several hundred from that equation.

MARTIN: How many of the 374 are organics?

AMSON: I would say more than half are inorganics. I'm not certain of the exact number.

VEITH: We're getting back to what I asked our Director a year ago. If we were going to establish a data base to draw predictions from using a Hansch approach or a similar approach, what would be the most useful endpoint to start with first?

The modellers even need a model to set priorities. In tomorrow's session, we can consider where this technique can be applied with the greatest likelihood of success. I doubt that the toxicity data that exists today will be useful to any great extent simply because of the wide variety of test conditions. A small variation in the method can destroy all correlations. There have been several attempts to come up with standard bioassay methods for 96 hour or 48 hour LC_{50} measurements. It would be useful to come up with some recommendations on what--I'm not looking for only a standard method, rather, what endpoints would be most useful to regulatory agencies, industry and to researchers. We are on the verge of much work being done on structure-activity correlations. If everyone starts out fragmentally with their own organism, they can culture in their laboratory and with their own test conditions, the data may not be directly comparable unless a lot more experiments are done. The recommendation for some uniformity for the purposes of the tests certainly would be a very powerful thing right now.

NEELY: I think it depends upon what you are looking at. If you are looking at Section 311, the 96 hour TLM is a good end value. When you get to 307, you are talking about toxic effluents which will be allowed to enter the streams and I don't think there is a single end value because end value will depend upon the particular location where the discharge is taking place. One of the things that bothers me, on seeing the preliminary regulations on 307 is that in the case of dieldrin, the effluent standard was set on the organism that was

most susceptible to dieldrin which happened to be the stone fly. I don't know if the stone fly is an integral part of the ecosystem where I live or not. If it is, obviously the stone fly becomes very important. If it's not, then I think a different organism should be tested. I think it's almost impossible for these gentlemen sitting in Washington or Ottawa to set a national standard that is going to apply throughout the country. I just don't see how they can do it.

ANDERSON: Maybe we should just totally overestimate using a simple, relative toxicity measure such as the LC_{50} to which one applies an unreasonable application factor. Having unreasonably overestimated the end-factor which you are searching for, put the burden of proof on those who are contaminating in making an adjustment to a reasonable but still safe level. That is, if they can justify higher levels through appropriate toxicity testing in the receiving waters, those levels are accepted by your agency for those conditions.

THURSTON: I certainly agree that looking at homologous series is a place to start. I especially agree with Drs. Veith and Anderson that it's only a starting place, but sooner or later, we have to try to test everything for good standards. With regard to the homologous series, if we made the mistake of testing ethanol in our martinis and found it safe today, and the next day mix our martinis out of methanol, we would be in trouble.

Concerning the stone flies studies and whether a national standard should be set on that, if we had more scientific input in Washington and Ottawa, we would realize that standards for the entire country may be less desireable than standards for major regions of the country. It is likely the investments of the additional scientific staff needed to categorize the regions and standards would be repaid manyfold by not setting unnecessarily stringent standard where they are not needed.

DE FREITAS: I will try to pick up what the last speaker said about leaving science and going to politics. From the standpoint of the workshop, we have to make a clear-cut distinction between the political and legal aspects first to see what reasonable set of procedures might produce, within the foreseeable future, a useful data base regardless of the existing legal structure. Then we need to work from there and see how we càn mold it within the existing legal framework. If we continually are talking about the two aspects, we are going in a frustrating circle.

HAMELINK: I would like to remind everyone of one thing. We understand DDT about as well as what mammaliam toxicologists understand what aspirin can do. None of us has mentioned this point of view of threshold. We departed from what mammaliam toxicologists and pharmacologists recognize that there are certain threshold levels. We can't justify infinite application factors. There will be truly some level at which threshold will occur and no effect will take place.

ANDERSON: I think you have to be very careful with the word "threshold". Sub-threshold does not mean there is no effect. It simply means there is no observable effect in relationship to the way you are examining the system. It could very well be that we are not establishing sensitive enough thresholds.

B . Summary Statements

During the last session the key participants attempted to: summarize the potentials of structure activity correlations; outline the areas in which the correlations should be pursued first; clarify the areas in which the correlations will not be of much use; and define research needs which may be transmitted to the Research Advisory Board of the International Joint Commission. During this discussion period many statements were made which may provide valuable information to both researchers and policy makers. The editors have attempted to summarize the comments of interest utilizing the phraseology of the participants to the greatest degree possible.

The Chairman prepared written statements with respect to these considerations and requested comments to formalize the general concensus of this symposium. These comments are summarized in the Chapter of "Conclusions".

> "The prediction of the biological activity of organic chemicals through correlations with structural parameters and biological activity of structurally related chemicals has been of considerable value to the pharmaceutical industry in the development of new drugs. The data presented and reviewed at this workshop

has shown that the structure-activity correlations can also be applied to studies involving aquatic organisms."

VEITH: Is there sufficient data to justify the use of structure-activity correlations in aquatic toxicity testing and bioaccumulation studies?

MARTIN: There are indeed limitations to this approach and inadequate data to wholly evaluate it. The drug industry is continuing to use, evaluate, and improve these methods, and is defining its own research needs. Quantitative structure-activity correlations could not as yet be considered the only approach; however, it can provide a valuable and different viewpoint.

LEO: Pomona College has on its files 1,600 sets of data which relate the effects of drugs to their chemical structure. The aqueous environment in this case was considered to be the blood stream. As data is gathered from toxicity tests, it will probably fit into a pattern. Since the data bases are not presently adequate, the predictions could only be considered as tentative.

ZITKO: The structure of a particular toxic substance and structure-activity relationships of related compounds should be determined. Data presented during this symposium encourages work with structure-activity relationships and if it works for drugs and their effects on mammals,

there is no reason why it should not work for determining the effects of toxicants on aquatic organisms. Structure-activity correlations could not be the only tool for toxicity determinations but this approach should be encouraged as much as possible.

VEITH: Based on what has been presented here the concluding statement could read:

> "that the data presented and reviewed at this workshop has shown that the structure-activity correlations can successfully be applied to studies involving aquatic organisms if adequate data bases are generated."

SCHAFFER: There would have to be a discrimination between aquatic systems in the laboratory and those in a natural environment. These correlations may not be applicable to aquatic organisms in both cases.

LEO: On extrapolating from laboratory to field conditions it should be noted that in actual applications pesticides are normally emulsified in the environment. Log P in the environment, therefore, may be somewhat lower than in the laboratory. But as the pesticide moves into the organism, log P becomes very similar to that determined in the laboratory.

VEITH: Can the statement be revised as follows:

"Data presented and reviewed at this workshop has shown that the structure-activity correlations have been successfully applied to toxicity testing with aquatic organisms?"

It is desirable to separate toxicity testing from bioaccumulation studies. In addition, bioconcentration factors in the laboratory correlate extremely well with those in the environment.

DR. FREITAS: The correlation between body burden at time of death and partition coefficients for a series of toxicants may not be as good as correlations between toxicity and partition coefficients due to (1) the relatively high toxicant concentration required in acute 48 hour or 96 hour tests and (2) impaired respiratory functions preceeding death. This would then suggest that uptake rate measurements, using nontoxic exposure levels over a short period (2-8 hours) could yield uptake rate constants that correlate well with partition coefficients and hence with toxicity results obtained in separate toxicity tests at higher toxicant concentrations.

LEO: It should be stressed that only within a given final mechanism of action is the partition coefficient going to be indicative. In other words, for membrane perturbation, partition coefficients are going to

decide which is going to be the most active for only those compounds that act that way. But it won't compare that with one that decouples oxidative phosphorylation. Only within the compounds that act within a specific mechanism will partition coefficients or sigma or some other physical-chemical parameter decide the most active ones. Partition coefficient appears to be very important in bioaccumulation; but the compounds that are collected may be acting by a number of different mechanisms. The mechanism will be the deciding factor in the toxicity response.

SEBA: The word "forecast" should be used instead of "predict". If we could forecast which compounds are likely to be hazardous, then we would define which groups require field and laboratory work.

KIMERLE: Our initial reaction to structure-activity correlations was skeptical, however, we are indeed finding an excellent relationship for groups of surfactants with correlation coefficients "r" of approximately 0.95. Such correlation should therefore have some utility and every opportunity should be utilized to look for these correlations. The legal implications of this approach should be considered only after an adequate data base is in existence.

BRANSON: Dow Chemical has evaluated toxicity of compounds over wide ranges of log P. Of interest is the invalidity of the standard 96 hour bioassay test to predict toxicity or hazards of high log P compounds in

the environment. Our laboratory has made a hypothesis for compounds which are very hydrophobic and possibly highly accumulative. Suppose we are concerned only with toxicity. If you measured the toxic values at 24, 48, 72, 96 and 120 hours, you notice that your LC values are dropping with time for high log P compounds. This essentially tells us that in 96 hours we're not really measuring anything that is very important to the environment. In fact, if we extended the life of that test longer we've got a more restrictive and a better assessment of the hazardous compound. We are uncertain of the critical log P value at which the 96 hour LC value drops off.

There is a potential research challenge to try to assign some kind of range of log P values where the 96 hour TLM is most appropriate. In fact, longer tests are required. One of the reasons of the importance is that sections 307 and 311 in the Water Quality Law are mostly confined to the 96 hour bioassay which is going to set the levels coming from the pipe. The acceptable levels are probably not low enough for high log P compounds and structure-activity correlations would give valuable information for such compounds. Furthermore for these same family of compounds with high log P, the water solubility is usually very low and LC_{50} is difficult to measure. Often the 96 hour LC_{50} values for the compound is higher than the solubility in water. What does this mean?

DR. FREITAS: Bioconcentration is a very difficult value to achieve experimentally and the use of uptake rate constants is recommended instead. The clearance rate constant would only be considered in long term testing. This type of correlation would economize in time and effort. The uptake rate is a tangent to the uptake curve and a short test may give an accurate uptake value. The bioconcentration factor is extremely difficult to obtain due to the increasing body burden of the fish and the increase in weight of the fish. If you define even one half of an uptake curve under ideal conditions, that can define both the rate constant of uptake and the rate constant of clearance without doing any clearance experiments at all.

MARTIN: In terms of drugs acting on mammalian systems, one can distinguish between biological activity and toxicity and there need not be any relationship between the two.

VEITH: In order to define the limitations of these correlations, could it be stated that:

> "The toxicity correlation can be applied to structurally related chemicals exhibiting the same mode of action with the same biological endpoint?"

ZITKO: (In response to a question on the use of the same correlations to predict sublethal and lethal effects.) Para-substituted are more toxic to fish than ortho-substituted phenols, however, ortho-substituted compounds are avoided more by fish than the para compounds.

This has been illustrated during the symposium and it is possible to predict toxicity of substituted benzene compounds, for example.

VEITH:

> "The parameters most useful in structure-activity correlations include the n-octonal/water partition coefficient, the Hammett σ constants, pK and the water solubility. Methodology for the calculation or measurement of these structural parameters of chemicals not included in existing data bases is adequate. The most comprehensive data bases for the prediction of partition coefficients and related parameters is that of the Pomona College Medicinal Chemistry Project at Claremont, California."

LEO: The data at Pomona College is available to anyone interested in this work. It should be noted that measurement of a partition coefficient is still the only way to be certain of its value and calculations still require much refinement.

SEBA: EPA has a data base for environmental information, TAD (Technical Assistance Data). This data base consists of 123 different categories for 700 compounds with information on toxicity values, clean up information, boiling points, etc.

MARTIN: We should recommend data bases which will be compatible, interchangeable and available to other groups. We also should recommend that within these data bases there be physical properties we think are important, so that we could search by log P for instance, and see what kind of toxicities come out.

VEITH:

> "Structure-activity correlative methodologies cannot presently predict the toxicity of mixtures of toxicants. There have been no demonstrated applications for questions other than where the relative toxicity of the individual chemicals are involved."

ANDERSON: I wonder if the participants here realize that two completely contradictory points of view have been presented and apparently jointly accepted as relevant at this meeting. One group has claimed that it is useful to correlate partition coefficients and structural design with toxicity and bioaccumulation. The other group has stated that the toxic unit methodology is effective in predicting toxicity. If one accepts the latter principle whereby constituents of a mixture regardless

of chemical structure are simply additive in accordance with their relative toxicity then one need not consider parameters such as partition coefficients etc. LC_{50} and application factors should suffice.

SEBA: The law reads that, until we have a better way of defining what we are working with, we will use this measure (toxic unit criteria). In other words, the law recognizes that it has a lot of limitation, but we had to have something so it was written in the absence of anything better. We are going to use this for the time being.

VEITH: The structure-activity concept could be used to determine relative toxicity of new organic compounds. The second more complex question is how to predict the toxicity of a mixture of these compounds given the relative proportions. It is difficult to foresee the application of structure-activity correlations to complex mixtures except in the simplest cases. Complex effluents are probably within the realm of predictive toxicology, but require different types of models.

ANDERSON: There had been at least two long term studies which have shown that the toxic unit concept is not applicable.

DE FREITAS: There is indeed a probability that prediction of multiple toxicity would not involve many more parameters than are already under consideration. There is a strong possibility of linearity of response.

MARTIN: Possibly we should define the necessary precision of the estimate. Perhaps an overall pattern should be the only objective and not a precise answer.

VEITH:

"The bioconcentration factors for organic chemicals are generally inversely related to water solubility and can be correlated to the n-octanol/water partition coefficient."

ZITKO: Solubility must occur before the compound can partition. In correlations between partition coefficients and toxicity, compounds may suddenly become non-toxic simply because the compound could not be solubilized. This was noted in studies with hydroxamic acid. It would be interesting to compare the uptake of chloro-biphenyls, which are crystalline, with liquid commercial PCB mixtures.

VEITH: Is the use of water solubility as a parameter to detect bioconcentration more appropriate than parition coefficients? Water solubility is operationally defined and this approach may be inadequate.

BRANSON: The accuracy of water solubility parameters is highly questionable.

VEITH: Within two or three years we should assess the applicability of structure-activity techniques both for the regulatory agencies and for industry in terms of screening and determining the toxicity of compounds without all the testing. There is a need for a rationale to estimate the toxicity of new chemicals due to the large number of organic compounds which are introduced to the market every year. There are inadequate data on existing materials, and testing all these

materials within a reasonable time period by bioassays alone is an impossibility.

MARTIN: One possibility is to screen the 5,000 compounds presently under a grandfather clause.

BRANSON: The implementation of structure correlations should be towards directing researchers towards the more significant toxicity tests.

VEITH: There is a basic research problem. There is also the matter of economics. If the curiosity isn't enough to try to apply correlations to determine "why" a chemical is more or less toxic than a structural related chemical, then the usefulness remains to simply decrease the number of tests that are necessary to make judgements.

SCHAEFFER: The participants should be reminded that correlation does not imply cause and effect relationships. It is important for the participants to bear in mind exactly what regression analysis is.

AMSON: We are losing sight of the difference between a receiving water standard and a standard which would apply to something that potentially may be spilled. I don't think under any circumstances we would establish a receiving water standard without having very definite data on the toxicity of that specific compound. On the other hand, with respect to something which has potential for being

spilled and may never be spilled, if we have five or six point on a curve and we drew a curve, and then we add something that is chemically similar, do we want to predict the relative potential hazard of a new substance on that curve? I think ultimately the answer is, "yes".

VEITH: Is it not a realization or conclusion that there is a need for information regarding relative toxicity of chemicals which are so numerous that it is impossible for us to test them all? Is it really believed that for the chemicals that are problems in the environment we should test them all?

HALL: In the detergent industry, we have a development program which is not unlike other industries. We create one, two or three new compounds which may be marketable each year. In order to arrive at those, we generate thousands of chemical structures yearly. Of these thousands of chemical structures, one is selected on the basis of performance at this point. There is a growing concern at Procter and Gamble, and I'm sure at other places, that these chemicals which are entering the environment will be important. People who are now making compounds are also beginning to consider their eventual fate in the environment. Our group concerning environmental effects is becoming more and more involved in the decisions which depend upon the selection of one of these structures.

We, therefore, have to make an environmental decision on many compounds or at least develop some trend so that we can more effectively predict which compounds have less environmental hazards. In this type of approach, we generate a regression curve for a group of compounds and, in this case, all of the compounds considered for one particular new product are very, very similar. They have only minor structural modification in order to increase efficacy. In these particular cases, I can see a tremendous application with this type of effort, where the selection of five or six compounds in what may be an "infinite" number of possible formula developments is made to get some toxicity and correlative data on these. We then are in a position to direct the synthesis of compounds which would be not only be effective as products, but also safe in the environment. I think this is very much like the "two-peak situation" in the phamacology industry where they want to have an effective compound and they want to keep the compound from causing toxic reactions. Our two peaks are efficacy in the product and not something in the environment, and that is a need.

SEBA: EPA has a need to effectively evaluate all the organic compounds in the environment. The biggest concern EPA has with organic compounds is their toxicity; both lethal and sub-lethal. Within the next three to five years the permit process will have to be repeated.

AMSON: There is also a need for scientists to keep communication open with the law makers in Washington, and more concern is required to apply research results to the real world. I would stress to keep the communication

open to keep people thinking how the other half lives.

ANDERSON: This sounds like a game of Russian roulette. While the gun is to our head, we are trying to develop models whereby we can predict in what chamber the bullet is. I think that we should, in relation to organic chemicals anyway, take a look at the finger that is on the trigger. Maybe the emphasis should not be to try to determine the toxicity for every individual organic chemical and set some sort of standards based on dilution for that particular chemical in the environment, but to simply eliminate it altogether.

AMSON: I'm not sure whose head and what the gun stands for.

ANDERSON: The head is, of course, the ecosystem. I guess we are part of that. The persons who are holding the gun are those who are contaminating the ecosystem with synthetic materials (organics).

AMSON: So what you are saying is actually the burden should be on them to prove they are not really doing damage to the environment.

ANDERSON: I think the burden should be placed on them to break down these organics. I think the models that we are presenting here serve the function of finding out the relative hazards which classes of chemicals or combinations of chemicals have. Using these models to define specific limits for particular chemicals and for all aquatic species would be foolhardy in my mind.

IJC REGIONAL OFFICE: There are at least two inventories of materials which are being used within the Great Lakes Basin. Structure-activity correlations might be used to determine which organic chemicals should be looked for in surveillance programs. The toxic organics which are now being measured in ongoing surveillance programs appear to be only four pesticides and occasionally PCB's.

VEITH: Structure-activity correlations can be best used to fulfill a need in industry and in regulatory agencies to screen organic chemicals for potential hazards in the environment. A data base of regression equations relating to toxicity or sub-lethal effects of related chemicals through structural parameters will greatly reduce the number of laboratory tests and may serve as an early warning in considerations for useage.

Petroleum plants may have effluents with 750 compounds. How toxic are these? There are so many homologous series in a petroleum discharge that it is senseless to think about determining toxicity of each. Moreover, there are at least 25 different categories of industrial effluents specified by the U.S. EPA, and this regulatory agency is currently under a dilemma of setting up and justifying appropriate discharge regulations. The use of the structure-activity models is one very promising tool to approach this problem.

C. Research Needs

VEITH: Structure-activity correlations, interpreted as predictive toxicology, will not address the problem of the toxicity of mixtures or the phenomenon of multiple toxicity. Clearly this is one field that needs a great deal of work outside the realm of structure-activity correlations. We have to make it clear that there is no way that we are going to predict the toxicity of mixtures with different modes of actions or unrelated parameters.

ANDERSON: In reality effluents contain more than one toxic substance and structural correlations for a particular series of substances may or may not be applicable in predicting the toxicity of complex effluents.

LEO: This approach can still be useful in dealing with mixtures. Although you can't predict that toxicity of any mixture, we might predict the lifetime in the composition of that mixture. At least it's better than just saying it's always going to stay the same.

VEITH: What work is required to enable the members of the panel to achieve their goals within a period of three years? Where is the research needed?

LEO: One very important goal in structure-activity correlation studies is to have a central pooling place in which pertinent information is collected, assessed and available to all investigators working in this field. Perhaps such a pool could exist within the structure of the EPA.

BRANSON: One research need is to evaluate the hazards of identified analytical anomalies found in samples.

Another is to identify a finite number of groups of homologous series (perhaps two or three), and determine toxicity relationships within chemical groups. We should be able to type-cast groups in terms of toxicity.

Bioconcentration studies should be considered most valuable. The fish is trying to tell us something, so let's listen to him. We should monitor fish and water routinely not for only four compounds. By a Delphi approach the list should be expanded to 50 materials which may be in the Great Lakes. The residues of these compounds should be correlated with the fat level in fish.

The fat levels pose a logical question on the significance of levels found in a perch or coho, etc. The law makers have to ask if that number is going to be useful and they are going to have to relate some sort of a deleterious effect on that level in the fish. We must link the deleterious effects with residues in the fish.

VEITH: The inventories mentioned should be investigated to determine if they are broken down by types of chemicals. This is an immediate need.

ANDERSON: Our preliminary studies on mixtures of toxicants would suggest that strict additivity is not a general principle. This is actually good news to those agencies who have been proceeding on the basis that you can establish permissible levels for individual toxicants. Nevertheless, there are certain combinations of discrete toxicants which do add, that is, they contribute to a common effect in proportion to each constituent's relative toxicity. These unique combinations should obviously be identified because of the hazard they pose in the aquatic environment. In setting standards for the individual toxicants one must consider their behavior in groups. It may be that in certain cases the toxic unit method suggested by Dr. Seba is effective. For instance many heavy metals appear to be additive.

However our model has also shown that in addition to strict summation there is another category termed interaction that may occur between certain chemical constituents of mixtures. Interaction could be such that the relative toxicity of discrete chemical constituents may be reduced. There is also the opposite situation whereby the relative toxicity is enhanced, that is, synergistic action occurs. These latter combinations of toxicants are real threats. I don't know of any particular way at the moment, to predict which combinations of toxicants synergize other than by diligent research on the multitude of various combinations as may exist in the environment. My model has also pointed out that there is a

third category of toxic behavior between constituents of a mixture. That is the category of independent action, whereupon a particular toxic constituent when below threshold (i.e. below the permissible level) will not contribute to the toxicity of the mixture. Our work suggests that many organic chemicals may fall into this latter category. It is this last category upon which I based my statement that it's good news to those in the regulatory agencies who have worked on the assumption that permissible levels can be established for individual toxicants. I think my model being broader in perspective than just simple additivity does offer a useful approach to appreciating multiple toxicity. I'm not saying, however, that it is the ultimate answer. I'm sure that there are many, many other points of view which will significantly add to our understanding of multiple toxicity in the future. But it is a step in the right direction.

There is a possibility for the application of partition coefficients and structural design models to the problems of multiple toxicity. Earlier I pointed out that if you accept, as a general principle, the phenomonon of strict addition then you are denying that you can class chemicals into groups based on structural design, partition coefficient characteristics, storage characteristics or whatever. But the model which I have been working on demonstrates that in fact there is more than one group of inter-actions and actions possible between chemicals. It would be interesting to see if in fact there is any relationship between partition coefficients and structural design and those groups of chemicals which are known to be strictly additive and those groups which demonstrate interaction, e.g. synergism and antagonism; and to those groups in which the constituents seem to be independent in their toxic action.

CHAPTER 15

CONCLUSIONS

The conclusions of the workshop can be summarized as follows:

1. The prediction of the biological activity of organic chemicals through correlations of structural parameters and the biological activity of related chemicals has been of considerable value to the pharmaceutical industry in the development of new drugs. The data presented and reviewed at this workshop has shown that the structure-activity correlations have been successfully applied to toxicity testing with aquatic organisms.

2. The structural parameters most useful in the structure-activity correlations include the n-octanol/water partition coefficients, the Hammet σ constant, the field and resonance constants, and the pK. The methodology for the measurement and/or calculation of these parameters is adequate. A comprehensive data base for structural parameters is available to researchers at Pomona College in Claremont, CA.

3. The structure-toxicity correlations can be applied to structurally related chemicals exhibiting the same mode of action and using the same biological endpoint.

4. The bioconcentration factors for organic chemicals in fish can be correlated to the n-octanol/water partition coefficient.

5. The structure-activity correlations are not likely to predict the toxicity of complex effluents even though the correlations may be valuable in multiple toxicity research.

6. There is a need in industry and regulatory agencies to screen large numbers of organic chemicals for potential hazards to the environment. The use of structure-activity correlations may greatly reduce the amount of laboratory testing required and serve as an early warning technique in a protocol for the use of toxic chemicals.

7. The uptake rates of chemicals significantly affects the death rate in acute toxicity tests. Consequently, the partition coefficient of the chemicals tested is important in selecting the LC_{50} endpoint (24, 48, 96 hr. etc.) for toxicity testing.

CHAPTER 15

CONCLUSIONS

CHAPTER 16

RESEARCH NEEDS

The data presented and reviewed at this Symposium have shown that structure-activity correlations have been successfully applied in testing with aquatic organisms. However, to apply this tool fully in forecasting the relative potential hazards of organic chemicals and in deriving water quality objectives, the following specific research needs must be addressed:

1. The toxicity of untested organic chemicals cannot be estimated reliably without an adequate data base of structure-activity correlations. Due to the foreseeable immediate application of this tool, it is recommended that a systematic protocol be developed to:
   a) categorize and select organic chemicals for testing based on their structural properties;
   b) prioritize the end points of toxicity testing and specify a standard method for use in the structure-activity correlations;
   c) direct the generation and compilation of data from the specified tests into the structure-activity data base.

   This protocol must recognize the variation of the LC_{50}-time relationship with the log P of this chemical.

2. It is recommended that an inventory of the chemicals produced and used in the Great Lakes Basin be made on a continuing basis. This inventory would include: total quantities of chemicals; a catagorization of major uses; structural parameters from existing data bases; and a streamlined compilation of pertinent data of toxicity or biological activity.

3. It is recommended that an extensive exploration of trace organic contaminants in the fish of the Great Lakes be initiated immediately, with reference to ongoing inventories of hazardous materials. When possible, the quantities of the contaminants should be measured in the water and fish populations to estimate bioconcentration factors in the Great Lakes environment and to relate these factors to the lipid content of fish and structural parameters of the chemical.

4. The above recommendations are concerned with immediate short-term problems with hazardous organic chemicals in the Great Lakes and other aquatic environments. A major, long-range research effort is urgently needed to address the problems of the toxicity of mixtures of hazardous chemicals, with emphasis on identifying the chemical properties which determine additive, synergistic and independent biological activity. This research is needed to develop predictive capabilities for complex multicomponent effluents.

5. This workshop reviewed and discussed the "toxic unit" concept which has already been implemented by the enforcement branches of regulatory agencies. There is a critical need to fully evaluate the "toxic unit" concept for regulating these discharges of hazardous chemicals.

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3. It is recommended that an extensive exploration of trace organic contaminants in the fish of the Great Lakes be initiated immediately, with reference to ongoing inventories of hazardous materials. When possible, the quantities of the contaminants should be measured in the water and fish populations to estimate bioconcentration factors in the Great Lakes environment and to relate these factors to the lipid content of fish and structural parameters of the chemical.

4. The above recommendations are concerned with immediate short-term problems with hazardous organic chemicals in the Great Lakes and other aquatic environments. In addition, there is a need to organize research to address the problems of the toxicity of mixtures of hazardous chemicals, with emphasis on identifying chemical properties which forecast additive, synergistic and independent biological activity. This research is prerequisite to effective regulatory strategies for complex multicomponent effluents.

5. This workshop reviewed and discussed the "toxic unit" concept which has already been implemented by the enforcement agencies in the regulatory programs. There is a critical need to fully evaluate the "toxic unit" concept for regulating these discharges of hazardous chemicals.

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TERMS OF REFERENCE — RESEARCH ADVISORY BOARD

1. As used herein, "research" includes development, demonstration and research activities, but does not include regular monitoring and surveillance of water quality.

2. The functions and responsibilities of the Research Advisory Board relating to research activities in Canada and the United States concerning the quality of the waters of the Great Lakes System shall be as follows:

(a) To review at regular intervals these research activities in order to:

(i) examine the adequacy and reliability of research results, their dissemination, and the effectiveness of their application;

(ii) identify deficiencies in their scope, and inadequacies in their funding and in completion schedules;

(iii) identify additional research projects that should be undertaken;

(iv) identify specific research programs for which international cooperation will be productive;

(b) To provide advice and consolidations of scientific opinion to the Commission and its boards on particular problems referred to the Advisory Board by the Commission or its boards;

(c) To facilitate both formal and informal international cooperation and coordination of research;

(d) To make recommendations to the Commission.

3. The Research Advisory Board on its own authority may seek analyses, assessments and recommendations from other professional, academic, governmental or intergovernmental groups about the problems of the Great Lakes water quality research and related research activities.

4. The International Joint Commission shall determine the size and composition of the Research Advisory Board. The Commission should appoint members to the Advisory Board from appropriate Federal, State and Provincial Government agencies and from other agencies, organizations and institutions involved in Great Lakes research activities. In making these appointments the Commission should consider individuals from the academic, scientific and industrial communities and the general public. Membership should be based primarily upon an individual's qualifications and potential contribution to the work of the Advisory Board.

5. The Research Advisory Board should work at all times in close cooperation with the Great Lakes Quality Board.

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— STANDING COMMITTEE ON THE SCIENTIFIC BASIS FOR WATER QUALITY CRITERIA

The Scientific Basis for Water Quality Criteria Committee of the Research Advisory Board has a mandate to:

1. Selectively assess the status of ongoing research related to water quality criteria for the Great Lakes to:
   a. Determine relationship of ongoing work to identified needs
   b. Identify opportunities for cooperative efforts.
2. Make recommendations to the Research Advisory Board concerning the above matters.

TERMS OF REFERENCE

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